For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex libris
universitatis
albertaensis

The University of Alberta
Printing Department
Edmonton, Alberta

Digitized by the Internet Archive
in 2020 with funding from
University of Alberta Libraries

https://archive.org/details/Sterenberg1969

THE UNIVERSITY OF ALBERTA

BIOLOGICAL POSITIONING IN ACCELERATED HUMAN

IRRADIATION

By

MARY EDITH STEVENSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

FALL, 1969

Thesis
1969(F)
231

THE UNIVERSITY OF ALBERTA

BIOLOGICAL DOSIMETRY IN ACCIDENTAL HUMAN IRRADIATION

By

© MARY EDITH STERENBERG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

FALL, 1969

THE UNIVERSITY OF ALBERTA

THE FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Biological Dosimetry in Accidental Human Irradiation" submitted by Mary Edith Sterenberg in partial fulfilment of the requirements for the Master of Science degree.

ABSTRACT

Within the last decade, much research has been directed towards examining the effects of ionizing radiations on the human organism. These efforts were hampered by a lack of suitable cells for tissue culture which would not become grossly different *in vitro* than *in vivo*. The discovery that peripheral leukocytes could be maintained in short-term cultures provided one means of correlating *in vivo* and *in vitro* cellular processes after irradiation. Consequently, attempts were made, notably by Bender *et al.*(several publications), to establish dose-effect relationships in terms of chromosomal aberrations using this peripheral leukocyte culture technique. The goal of this research was the determination of "coefficients of aberration production" which could be fitted to a general quadratic or linear function; this equation could be used to determine the dose received where accidental irradiation of an unknown dose had occurred. In view of certain inconsistencies in the chromosomal aberration method of biological dosimetry, the peripheral leukocyte culture method has been employed to establish dose-effect relationships for cellular aberrations (including changes in frequencies of morphological types) mainly by Weijer *et al.*(several publications).

This comparative study of the estimations of radiation dose by the two methods of biological dosimetry mentioned above, was facilitated by the occurrence of an accidental human exposure to X-irradiation. The chromosomal aberration method yielded the information that the bone marrow was probably irradiated, and con-

firmed the dose indicated by the film badge dosimeter. The cellular aberration dosimeter is of more limited value for this case because of the time interval of six to seven weeks between irradiation and sampling; however, it serves to establish a lower limit to the possible exposure. (60R). The upper limit established by the chromosome aberration method, is approximately the premise of preservation of aberrations in vivo may have limited validity for both methods of biological dosimetry; and it is within this limit that the determination of unknown doses of accidental irradiation may be considered accurate.

The chromosome associations observed are not a consequence of radiation damage, but represent a phenomenon frequently observed in mitotic metaphase preparations.

Generally, these associations in themselves are not indicative of any genetic abnormality; in this case, there is no outward expression of any abnormality.

ACKNOWLEDGEMENTS

I am indebted to Dr. Weijer for his continuing advice and encouragement during the course of this study. My thanks are extended to the volunteer blood donors who contributed for the experiments which were a preliminary to this work. The assistance of Mr. Ramesh Bhambani in preparing the chromosomal aberration material is gratefully acknowledged.

This study was supported by a Post graduate Scholarship from the National Research Council of Canada.

TABLE OF CONTENTS

LIST OF TABLES, FIGURES AND PLATES

INTRODUCTION

The problem of accurate biological radiation dosimetry, as applied to human exposure, represent a relatively new field of research in radiobiology. While radiation cytology and cytogenetics date back to the work of Sax in the 1930's and biological dosimetry of radiation effects in plant cells had been carried out by Lea by 1955, it was only after 1957 that animal tissue culture became involved. For human exposure, radiation dosimetry has been carried out using two methods: (a) studies on induced chromosomal aberrations and (b) studies on gross changes in cell morphology. For both types of study the lymphocytes of the peripheral blood have been widely utilized.

The actual test of accuracy of a biological dosimeter lies in its application in radiation incidents. Infrequently, a situation arises where a radiation accident has happened, in which the physical dosimetry is known in terms of film badge exposure. In these cases it is possible to culture peripheral blood leukocytes and evaluate the dose received using a biological dosimeter based on either nuclear (i.e., chromosomal abberration) or morphological (i.e., cellular aberration) studies.

The present investigation is concerned with one such accident. In August of 1968, a situation arose where a physical dosimeter registered, on two separate occasions, what were thought to be improbably exposures to ionizing radiation. A complete cytogenetic analysis has been carried out using both parameters

for dosimetry, and the dose estimates obtained have been compared with the dose indicated by the physical dosimeter.

In addition, chromosomal associations probably not related to the radiation exposure were revealed by cytogenetic analysis. A short discussion of these associations, with illustrative plates is included in this study.

LITERATURE REVIEW

By the early 1960's several workers had already established the incidence of nuclear aberrations resulting from exposure to ionizing radiation in animal tissue (*in vitro*) and in experimental animals (*in vivo*). A correlation between frequencies of aberrations and does was found to exist not only between animals tested *in vivo* but also when comparing *in vivo* with *in vitro* results. (Bender, 1964).

Biological dosimetry of human exposure using chromosomal aberration data was not feasible prior to 1959 because the cells used commonly originated from long-term cultures in which the cell characteristics had altered greatly from the original primary explant. Hence, data from these experiments could not be expected to approximate *in vivo* chromosomal aberration data. Furthermore, no reasonable method was available for providing metaphase preparations in which the chromosomes and their aberrations were clearly distinguishable. The methods developed for short-term leukocyte culture (Hungerford *et al.*, 1959) and for obtaining prefectly spread metaphases from these (Moorhead *et al.*, 1960) were the first steps toward using a human cell system in chromosomal aberration dosimetry. These short-term cultures, irradiated immediately after sampling (i.e. before separation of leukocytes), could be expected to approximate most closely *in vivo* conditions; furthermore, it now became possible to obtain *in vivo* results from blood withdrawn from therapeutically irradiated patients; thus providing an *in vivo* - *in vitro* comparison of the same type

of cell.

The original studies on rates of chromosmal aberration production, in experimental animals *in vivo* and animal tissue *in vitro*, as well as on human diploid solid tissues *in vitro*, and on human peripheral leukocytes (Bender, 1964) were based on the assumption that breakage was a linear function of dose received; that is, a measurement in terms of breaks/cell per rad (Bell & Baker, 1962; Bender & Gooch, 1962a). When it became apparent that total breakage increased by a factor greater than the first power of dose, the more accurate measure of damage, the "coefficient of aberration production" (as developed by Lea in 1955) was revived. For different types of aberrations characterised by different kinetics of production, different coefficients had to be derived. For deletion type aberrations which are single-hit phenomena, in the Target theory (Lea, 1955) an increase in frequency as the first power of the dose was observed, while exchanges or multi-hitphenomena increased approximately, as the square of the dose (Bender & Gooch, 1962a; Bender, 1964, 1968; Bender & Barcinski, 1968; Norman *et al.*, 1964, Norman & Sasaki, 1966, Norman *et al.*, 1966).

Bender and Gooch (1962a) derived an expression which related total breakage (for chromosome-type aberrations) to dose. This was

the quadratic equation $Y = a + bD + cD^2$ (1), where Y = total yield of breaks, a = spontaneous aberration frequency, D = dose in Roentgens, and b and c are the coefficients of production for one and two-break aberrations. In more recent publications these authors and others (Norman, 1964) have favored a bipartite formula for calculating yield of aberrations rather than breaks, for both chromosome and chromatid-type breakage. For one-hit aberrations of either chromosome or chromatid-type, the linear function $Y = a + bD$ (2) has been applied, using the different values of a and b derived for chromosome or chromatid-type aberrations (Bender and Gooch, 1963, 1964; Norman, 1964). For two-hit aberrations, the classical expression $Y = cD^2$ (3), where the spontaneous frequency a is zero, has been used.

For chromosome-type aberrations (one-hit events only) the linear expression $Y = a + bD$ is used, employing the coefficient $b = 0.11 \pm .01 \times 10^{-2}$ deletions/cell per R (Bender and Gooch, 1962a). For yield of two-hit aberrations, the coefficient $c = 0.52 \times 10^{-5}$ dicentrics and rings/ cell per R^2 is a mean value of a range of values (given in Table I) for the yield of dicentrics and rings. For total yield of breaks these coefficients are used in the quadratic function (1). The spontaneous rate of breakage a, for chromosome-type breakage, has been established as 0.0023 breaks per cell.

The linear function for yield of chromatid aberrations also becomes the expression for total breakage yield for this class of aberration, because two-hit aberrations are not easily de-

tected. The values assigned to a and b were set at 0.032 breaks/cell and 0.0031 breaks/cell per R, respectively (Bender and Gooch, 1963).

For dosimetry of human radiation exposure, the expression $Y = cD^2$ may be of more value. Most of the aberrations in lymphocytes irradiated in vivo are of the chromosome-type; and of these, the accurate scoring of deletions involves preparing karyotypes in order to detect symmetrical exchanges or terminal deletions (if fragments have been lost in preparation). Ring and dicentric chromosomes, however, can be scored with greater ease.

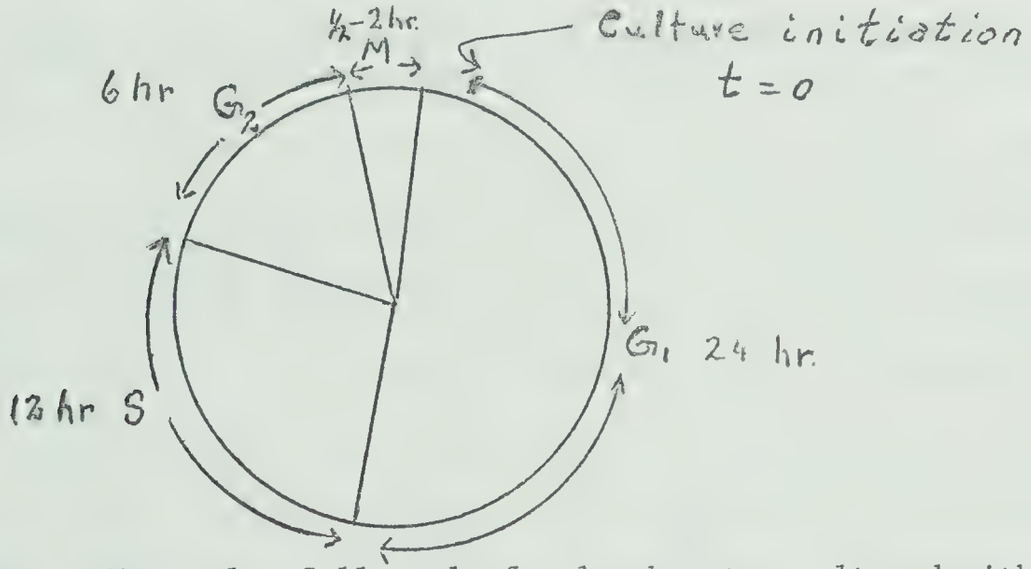
The lymphocyte system is well suited to this type of study, not only because of the relative ease of preparation of chromosome spreads from short-term cultures, but also because of the peculiar cell cycle kinetics of the lymphocytes. This cell was originally termed an "end" cell in the leukocyte series, not normally capable of further division in vivo, later it was suggested that this was a potential stem cell, which could be activated by an immunological stimulus (Bloom & Fawcett, 1962).

The peripheral blood was recognized to support two lymphocyte populations, "large" and "small" lymphocytes distinguished on the basis of cell diameters. The latter has recently been tentatively sub-divided into two populations, one with a short life span of several weeks; and one which is considerably longer-lived, possibly up to several years duration (Ottesen, 1954; Hamilton, 1954, Buckton & Pike, 1964).

In tissue culture, under the influence of the non-specific mitogen phytohaemagglutinin (PHA) or one of several specific antigens

(Robbins, 1960, Nowell, 1960) the small lymphocyte can be induced to undergo "transformation" to a large "blast" cells; these in turn go through a period of DNA synthesis, and a mitotic division to produce cells which resemble the original small lymphocyte. These conclusions were based on morphological studies and on tracer studies using labelled thymidine (Gowans, 1962; Porter & Cooper, 1962; Yoffey, 1963; Tanaka et al., 1963; Caron, 1968).

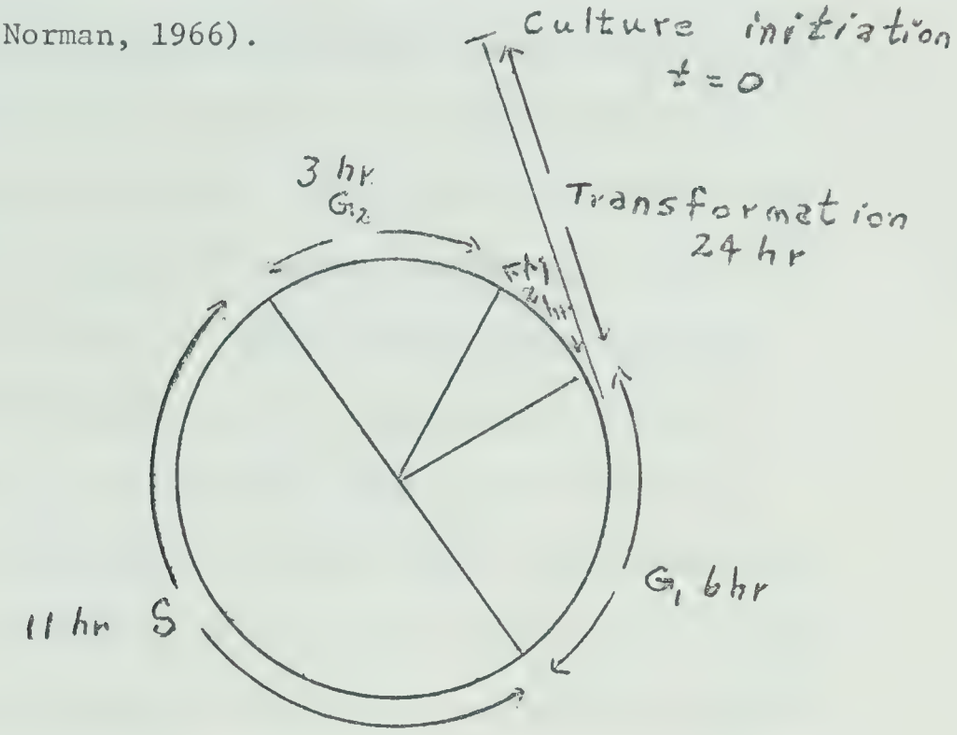
It is generally held that the small lymphocyte in the body is in the G_1 period of the cell cycle. Through tracer studies, the cycle has been established as indicated in text-figure 1. (Bender & Prescott, 1962; Cooper, Barkhan and Hale, 1963).



Text - Figure 1. Cell cycle for lymphocytes cultured with phytohaemagglutin, (Bender & Prescott, 1962).

Other investigators support the view that the small lymphocyte in vivo and the cell which undertakes blastogenesis in vitro are subtly different; that is, a new cycle is introduced at the time transformation is completed. Thus, the small lymphocyte may be in the G_1 phase in the body, but the new cycle introduced after transformation proceeds with slightly different times spent in each phase;

and certainly with a much shorter cycle, as shown in text-figure 2. (data from Sasaki & Norman, 1966).



Text-figure 2. Cell cycle for lymphocytes cultured with phytohaemagglutinin. (Sasaki and Norman, 1966).

In either eventuality the lymphocyte does not normally divide *in vivo*, and any injury to the chromosomes will be preserved until division is induced, provided the injury is not immediately lethal to the cell and does not cause too great a shortening of the life span of the cell, resulting in eventual death. Thus, the cells may be cultured some time after irradiation, and aberrations will be detectable in the cultured cells, provided the cultures are arrested at the first metaphase in culture (Buckton & Pike, 1964).

That lymphocytes are in the G_1 stage in the blood can be confirmed by the method of deliberate timing of irradiation of cultures. Whole blood irradiated immediately after sampling yielded only chromosome-type aberrations after 54 and 72 hours

in culture; while cultures irradiated at 66 hours after sampling and harvested 6 hours later showed primarily chromatid aberrations. Consequently, the lymphocytes must have been in the pre-replication stage in the first case while in the second, replication must have occured prior to irradiation (Bender and Gooch), 1962a, 1963). In both experiments the rates of induced aberrations coincided. From these results, the investigators concluded that all the lymphocytes, being in G_1, were uniformly radiation sensitive; this conclusion was borne out by the uniformity of the aberration data (Bender & Gooch, 1963, p. 113). Later studies on the immediate effects of irradiation appear to confirm that there is no apparent difference in the immediate radiosensitivity of the large and small lymphocytes (Neff & Cassen, 1968).

Thus, the lymphocyte system is advantageous because no correction need be applied for the bariable radiosensitivity of different stages of the cell cycle; the lymphocytes are "automatically" synchronized in G_1.

In the earlier studies done using cultures harvested after 72 hours of incubation, the investigators assumed that at this culture level the cells were all in the first metaphase post irradiation (Bender & Gooch, 1962a). However, recent work has shown that as many as 70% of the mitotic cells may be in second division at this time. (Sasaki & Norman, 1966). Bell & Baker (1962) harvested cells at 100 hours which were irradiated at various times during this culture period. They found no loss

of aberrations due to intervening mitosis; but they admitted "it would appear more proper to score aberrations at the first post-irradiation metaphase, as such loss or alteration of aberrations during the first and subsequent mitoses makes later scoring inaccurate." (p. 348). The implications are that a loss of unstable aberrations (i.e., dicentrics, rings, and fragments) may occur, either because the cell is unable to proceed through the first division or, although division takes place, these aberrations are excluded from the resulting daughter cells. For example, chromosome-type deletions could not be scored without a karyotype analysis. Norman & Sasaki (1967) estimated that 70% of acentric fragments were lost in the first division after irradiation and dicentrics were lost at a rate of 50% per cycle, in vitro. (In vivo, cells with unstable aberrations decline in numbers over a period of years, but there is no evidence that this is due to division in vivo (Buckton and Pike, 1964).) It can seem that the early estimates of coefficients of aberration, if based on metaphases, from the second division post-irradiation probably led to very conservative estimates of breakage. Reference-Table I (see Appendix I) gives the range of values assigned by various workers as aberration coefficients.

Paralleling these aberration studies an attempt has been made to establish a standard dose-effect relationship using other parameters of the leukocyte culture method. These are gross morphological changes which have long been recognized as symptomatic of radiation injury. These phenomena, including cellular pyknosis,

binucleate cells, lymphocyte depression, and others, were described fairly early in the literature in regard to radiation sensitivity of the lymphocyte series, but interest was shifted to the chromosome aberration studies already described, when adequate methods of preparing material for these studies were devised.

Binucleate cells were described by Ingram & Barnes as early as 1949, in humans previously exposed to continuous total body irradiation from a cyclotron. Later, these observations as a predictable occurrence in irradiated blood, were confirmed by Dobson & Chupp (1957) for human exposure, and by Ingram (1960) for experimental animals. Weijer et al., (1963), in studies using 32P, 35S, and X-rays as radiation sources, showed that the production of binucleates varied according to the type of radiation; but for all three types, the frequency of binucleate cells was a function of radiation dose and incubation time. The origin of binucleate cells remains a mystery. Although there is evidence that an appreciable number of these cells are of a lymphocytic nature, some exhibit in stained preparations, a characteristic granulation reminiscent of the eosinophilic neutrophil.

It has long been known that the small lymphocyte is one of the most radiosensitive cells in the body. In in vitro studies, pyknotic nuclei appear in increasing numbers during the first 4 to 6 hours after in vitro exposure to 100 to 400 rads (Schrek, 1961; Trowell, 1952). Very low doses produce no immediate pyknosis but a shortened life span is probably indicated by the decrease

in survival time (Schrek, 1961). It has also been observed that there is a rapid fall in the peripheral lymphocyte count after *in vivo* irradiation (Jacobson *et al.*, 1950), but it is assumed that this decrease is not due to immediate killing and lysis of the cells. The number of pyknotic cells arising in blood smears taken immediately post-irradiation does not account for this great immediate lymphocyte depression observed in *in vivo* studies. In *in vitro* studies there is no increase in pyknotic cells immediately post-irradiation; in the cultures, pyknotics appear after one to two days. Trowell (1952) suggested there may be recognition of injured or dead cells and removal to some organ in the incubation pathway for destruction or repair. Alternatively, there may be an immunological reaction elicited by irradiation, in the tissues, to which the lymphocytes immediately respond and leave the blood. Investigations have been carried out to determine whether there exists a differential sensitivity of lymphocytes towards ionizing radiation. It has been assumed that the small lymphocyte is more sensitive to ionizing radiation (Everett *et al.*, 1964) however, Neff and Cassen (1968) have found that during the first eight hours after acute irradiation, there is no difference in sensitivity. Berke *et al.* (1969) found that the relative numbers of large and small mononuclear cells were changed, that is to say, that the ratio of large vs. small mononuclear cells was depressed for three days after irradiation, but rose during the subsequent period. For fractionated irradiations, they found transient depressions lasting two to three days after

the initial doses, after which a gradual rise above the control values was seen. Shiels (1962) reported an increased ratio between large vs small lymphocytes with continuous exposure to irradiation (i.e., occupational exposure). It would appear that for one-dose exposure, during the time of the most critical mononuclear depression and shift in the ratio of large vs small lymphocytes, the large lymphocytic cells are being removed more rapidly (see Berke et al., 1969); while for occupational exposure the opposite would appear to be true. It is not at all clear from the limited and conflicting data what underlies the rapid depression and which population of lymphocytes is affected after radiation.

Pyknotic cells do not usually appear in smears of healthy blood, but can be found under special physiological and pathological circumstances (Ingram, 1960). Thus there appears to be rapid removal of dying cells in healthy blood, which seems to confirm the idea of rapid removal of injured or dying cells from irradiated blood in vivo. On the other hand, Schrek (1961) found that in blood irradiated in vivo and then cultured, the survival time of lymphocytes (S 10%) was shortened compared to controls. Hence, there must be physiologically injured cells circulating in the blood which produce this change in vitro and probably a shortened life span in vivo. In peripheral blood cultures, using normal or irradiated blood, pyknotic cells are always present in numbers which reflect the severity of separation procedures and the culture conditions, (e.g. radiation) as well as the normal death of ageing cells and their

accumulation in the culture. Schrek (loc. cit.) found that unirradiated cells died by the same process as irradiated ones, i.e. by vacuolization and nuclear pyknosis. "Moderate doses of X-rays did not kill lymphocytes but only accelerated normal death." (Schrek, 1961).

In the same studies already mentioned, Weijer et al. (1963) showed that the incidence of pyknotic cells in x-irradiated cultures was correlated with dose and culture time, a relationship such as "an increase in the dose by a factor of four will give rise to an increase in the number of pyknotic cells by a factor of approximately two at equal culture times. "(Weijer et al., 1963, p.2)

In view of this information, Weijer has attempted to construct a reliable dosimeter using cultured irradiated blood, noting the dose-effect relationships with regard to gross morphological changes in the lymphocytes. The parameters used and the data obtained are given in Reference-Table II, (see Appendix I). The standard curves fitted to these data are given in Figures 1-4, (Appendix I; Weijer & Weijer, 1969, in press). A further modification to this method, using the criteria of 'early' and 'late' damage has recently been suggested by Weijer. A short discussion of his theory and its' application in this radiation incident are included in Appendix II (Weijer, personal communication).

MATERIALS & METHODS

The time elapsed between accidental irradiation and sampling of blood is of critical importance in evaluating biological damage by means of the biological dosimeters. A short account of the events which transpired before blood samples for cytogenetic examination in the analysis of the underlying case were received, has been included (Weijer & Weijer, 1968).

The Radiation Exposure Report, which is sent every two weeks to all personnel using film badges, (and which is calculated on the basis of two weeks exposure of photographic emulsion during normal working conditions) dated August 16, 1968, stated an initial exposure of 35,000 mR for the 14th two week period 1-15 July, 1968. The information was not received until August 20, 1968, by Telex, due to postal difficulties. The genetics department was apprised of the case on August 21; blood samples were obtained for culture on August 26. On that same day notification was received (and confirmed on August 27 by the radiation exposure report) that an exposure of 60,000 mR had been recorded for the 15th two-week period. Thus the second exposure occurred sometime during the period July 16 - 31, 1968. Blood samples for a second culture were obtained September 3, at least six weeks after the initial radiation exposure.

There is no evidence to indicate whether these doses were received on two single occasions or continuously over each period of two weeks.

1. Method for Coverslip Cultures

Two 10 ml tubes of blood obtained by venous puncture were allowed to separate by sedimentation for one hour at 37°C. The only additive was 0.1 ml of the anticoagulant heparin (1000 IU/ml). The tubes were then centrifuged for two minutes at 500 rpm in a clinical centrifuge. Serum and leukocytes were removed by aspiration and cultures were set up using 65% Medium 199 (BBL), 35% homologous serum, and the additive antibiotics penicillin (100 IU) and streptomycin (100 mcg). No phytohaemagglutinin (PHA) was added to these cultures. Two ml aliquots were dispensed in Leighton tubes with glass coverslips (No. 1, 10.5 x 50 mm, Bellco Glass Inc.).

The cultures were sealed and incubated for 72 hours at 37°C. The coverslips with adhering cells were then removed and fixed in methanol for 2 minutes; stained through the Harris - haematoxylin - Eosin procedure, dehydrated through the ethanol-xylol series, and mounted on clean glass slides in Canada Balsam. These slides were examined for evidence of radiation damage in terms of induced changes in cell morphology. Cell counts were made using a Leitz Orthoplan microscope with a 90x oil immersion, bright field objective and 10x oculars. Photographs of representative cells were taken using the same optics, with a Leitz Orthomat camera and high contrast film. Prints were made on Vee-Cee Rapid N.S.W. paper.

The frequencies of cells exhibiting lethal or sub-lethal damage were compared with standard frequencies experimentally established for different doses of ionizing radiation by Weijer & Weijer (1969).

2. Cultures for Chromosome Analysis

Twenty ml of heparinized blood, obtained as described in subsection 1, were sedimented using 3% gelatin in a ratio of 3 parts blood: 1 part gelatin, according to the method of Coulson & Chalmers (1964), except that PHA (General Biochemicals, 0.2 ml/10 ml blood-gelatine mixture) was added. This was allowed to sediment for 1/2 hour at 37°C. The blood-gelatin mixture was then centrifuged at low speed (500 rpm) for 3-5 minutes. The supernatant (gelatin plus plasma plus lymphocytes) was aspirated and the cells concentrated by centrifugation at 800 rpm for 5-10 minutes. The supernatant was discarded and the cells were resuspended in 12 ml Medium 199 (BBL) with 4 ml Fetal Calf Serum (BBL), 0.3 ml phytohaemagglutinin and penicillin-streptomycin added. This was divided to make two cultures, in Falcon T-30 plastic culture flasks (B-D Laboratories). Cultures were incubated at 37°C for 67 hours after which 1.5 ml of .008% colchicine (0.008g/100ml) were added to each culture. After 5 hours incubation with colchicine, the cultures were transferred into conical centrifuge tubes and centrifuged at 800 rpm for 5-10 minutes. The supernatant was discarded and the cells were resuspended in 5 ml of prewarmed 1% sodium citrate. After 10 min. in the hypotonic, the cells were centrifuged for 10 min.; the supernatant was aspirated off, and the cells were fixed in freshly made acetic methanol, (1:3) for 30 min. After 3 washes in fixative, with intervening centrifugation, the cells were resuspended in about 1 ml. of fixative. Slides

were prepared and stained by the modified Feulgen technique (Jacobson, 1968; Weijer and Weisberg, 1966). Coverslips were applied over water mounting medium (Gurr's). When dry the slides were examined for well-spread metaphases. Approximately 100 different cells were examined and photographed using bright field, oil immersion (90 x objective) and phase-contrast, oil immersion (100 x objective) optics, both with 10x oculars; photography was done using a Leitz Orthomat camera with xenon-illumination, and 35 mm high-contrast film. The paper used for prints was Vee-Cee Rapid N.S.W.

The metaphase plates so produced were analysed for chromosomal aberrations. Dosimetry was carried out according to the method of Bender & Gooch (1962a), using aberration coefficients which have been updated since that time (Bender, 1968, Norman, 1967). In addition, karyotypes were prepared from selected metaphases to identify the chromosome groups that were involved in the above-mentioned chromosome associations.

RESULTS

I. Aberrant Leukocyte Morphology in vitro

A preliminary examination of the coverslip cultures of blood from the accident victim revealed aberrant cellular forms, in frequencies well above those found previously in controls (0R) of cultured lymphocytes irradiated with 0-400R of X-rays (Weijer, 1969). Elevated numbers of pyknotic and 'blast' (premitotic) cells were evident even before counts were made; similarly, there were many more living neutrophils present in these cultures after 72 hours (Plates 1-3, Appendix I), than in cultures or irradiated blood harvested after the same time in culture (Weijer, 1969).

Precise evaluation of the cultures initiated on August 26 and on September 3, 1968, using the parameters of Weijer (1969, Reference-Table II, p. 35) yielded the values given in Table I. The data for pyknotic cells, surviving lymphocytes, and surviving neutrophils are based on three separate counts each of 1000 cells, scoring these three cell types simultaneously. (It was found that scoring only one type of cell per 1000 cells prejudiced the count in favor of that cell type,) For the blast and premitotic cells, three separate counts were made to a total of 1500 cells (August 29) and two separate counts to a total of 1200 cells (September 6). The binucleate cells were scored per 1500 cells (August 29) or per 1000 cells (September 3).

From data on pyknotic cells, surviving lymphocytes and neutrophils in culture (initiated at six weeks post-irradiation),

TABLE I

Cell Survival and Cellular Aberration Frequencies
after Accidental Irradiation

		August 29, 1968 Count Number				September 6, 1968 Count Number			
	Cell Type	1	2	3	Mean %	1	2	3	Mean %
1.	Pyknotic	258	219	377	29.5	198	167	216	19.4
2.	Surviving Lymphocyte	610	666	536	60.4	681	739	673	69.8
3.	Neutrophil	132	115	87	11.1	121	94	111	10.8
	Total cells	1000	1000	1000		1000	1000	1000	
4.	Blast & Premitotic	241/500	221/500	218/500	45.3	230/700	194/500	----	35.3
5.	Binucleate	11/500	7/500	22/500	2.7	9/500	20/500	----	2.9

(Cell type (2) includes types (4) and (5).)

it was calculated that the residual biological damage was equivalent to biological blood damage resulting from a total body dose of 30R ± 5R. In cultures initiated one week later, the residual biological damage was assessed as equivalent to a total body dose of 25R ± 5R. This represents a decrease of 5R over a one-week period. Assuming a linear relation between decay of apparent damage and time after irradiation, and assuming the accident occured 6 weeks prior to first sampling the total body dose received must have been at least 30R + (5R x 6 weeks) = 60R. This is certainly a minimum estimate since the relation is not a linear one, but has a near vertical component in the first 2-3 days post-irradiation, indicating rapid changes in the assessable damage. The second component has a gradual slope, indicating a slower decrease in decay of assessable damage (see Figure 5a, Appendix I).

II. Aberrant Chromosome Morphology *in vitro*.

TABLE II

Chromosomal Aberrations in 100 Metaphase Plates from 72-hour Cultures

(6 weeks post-irradiation)

Aberation Type	Tabulator J.W.	M.S.	Mean
1. Chromatid Deletion	27	36	32
2. Chromosome a) Deletion	13	10	11.5
b) Exchange	4	2	6
c) Ring	2	5*	4.5*
d) Dicentric	2		

*Ring and dicentric chromosomes counted as one group

The results of this study are given in Table II. Independent counts were made by two investigators, using the same photographic prints of 100 metaphases. Representative metaphase plates are shown in Plates 4-15, (Appendix I). Only those aberrations which were quite clearly evident were tabulated. All doubtful ones were scored as normal. Hence, the estimates given in Table II can be considered as minimum values.

The large number of chromatid breaks observed necessitates a classification and analysis of the data for a) chromosome-type aberrations and b) chromatid aberrations. The quadratic function (1) was derived by Bender and Gooch (1962) to calculate total breakage when the chromatid breakage rate is extremely low; (i.e. primarily for chromosome type breakage). Furthermore, calculation of the dose received by the two methods mentioned above, provides a check on the accuracy of either method, and establishes a limit of values between which the actual dose received should be situtated.

A) Chromosome Type Aberrations.

1) The total yield of deletions was 11.5/100 cells. Using $Y = a + bD$ (2), with a = .0023 breaks/cell and b = .0011 deletions/cell per R,

$$0.115 \text{ deletions/cell} = .0023 = .0011D$$

$$D = 102 \text{ R.}$$

2) The total yield of ring and dicentric chromosomes was 5/100 cells. Using $Y = cD^2$ (3), where $c = 0.52 \times 10^{-5}$ dicentrics and rings/cell per $R,^2$

$$0.05 \text{ dicentrics and rings/cell} = 0.52 \times 10^{-5} D^2$$

$$D = 98 \text{ R.}$$

3) The total yield of chromosome type breaks* was 21/100 cells *(total deletions plus twice the total of rings and dicentrics) Using $Y = a + bD + 2cD^2$ (1), where a = 0.0023 breaks/cell, b = 0.0011 deletions/cell per R, and c = 0.52×10^{-5} dicentrics/cell per R^2,

$$0.21 \text{ breaks/cell} = 0.0023 + 0.0011D + 2(0.52) \times 10^{-5} D^2$$

$$D = 100 \text{ R.}$$

B) Chromatid - Type Aberrations.

The total yield of chromatid-type deletions was 32/100 cells. Using $Y = a + bD$, where a = 0.032 breaks/cell and b = 0.0031 deletions/cell per R,

$$0.32 \text{ deletions/cell} = 0.032 + 0.0031D$$

$$D = 93 \text{ R.}$$

The combined results of cellular and chromosomal aberration studies establish a total dose of X-rays between 60 R, minimum, and 102 R, maximum. The calculations using chromsome and chromatid aberrations formulae (1),(2), and (3) give nearly identical dose estimations. The dose indicated by the physical dosimeter is within the limit (60R-102R) indicated by the biological dosimeters, and is approximately the same as the dose indicated by the nuclear aberration data.

III. Chromosome Associations

The chromosome associations in 100 metaphase plates were classified into groups and the results are given in Table III.

TABLE III

Type of Association	Number of Cells	Number of Associations
1. Association of any chromosomes	70 cells	107 associations
2. Any association involving D or G chromosomes	67 cells	99 associations
3. Specific association of two D and one G chromosomes	32 cells	33 associations
4. All associations other than (3)	57 cells	74 associations
5. Associations involving D or G chromosomes other than (3)	57 cells	66 associations

In 67% of the cells an association of at least one chromosome of group D (13-15) or group G (21-22) with another chromosome of these groups or with any other chromosome, was observed. Furthermore, in 32% of the cells, a specific association of two group D chromosomes and one group G chromosome was observed. Only 7 associations were observed which did not involve chromosomes of these two groups. Associations in which these D - or G - group chromosomes participate invariably show involvement of the short arm of either or both D and G group chromosomes with another short arm of a D or G group chromosome, or with either arm of a chromosome in the metacentric or sub-metacentric groups (see Plates 4-17, Appendix I).

DISCUSSION

The biological dosimeters of Bender (1962a) and Weijer (1969) have been employed in analysis of this case, to confirm that the dose registered by the physical dosimeter was an actual exposure, probably to the entire body.

Aberrant Leukocyte Morphology.

In the experimental determination of dose-response relations, certain of the parameters used are merely observational and not statistically significant. Furthermore, different treatments may give the same results as irradiation for these parameters. The appearance of binucleates is a common phenomenon, although in cultures of normal blood the cells are rare. Dobson and Chupp (1957) state that these cells appear in lymphatic leukaemia, in hepatitis, and may appear after heat treatment in animals. Although binucleate lymphocytes have been reported in the bloodstream several years post-irradiation (Ingram, 1960), the binucleate cells often seen in vitro immediately after irradiation appear to be of a different origin. The binucleate of Ingram is of unquestionable lymphocytic origin. The binucleate appearing in in vitro cultures can be either lymphocytic but more commonly neutrophilic with typical eosinophilic characteristics.

In the data of Weijer (1969) the frequencies of binucleate cells have large standard deviations that render this parameter, by itself, unsuitable for a dose estimation. In the accident case under consideration, the paucity of binucleate cells led Weijer to suggest that a total body exposure might not have been involved.

Another observational parameter (not used here) is the phenomenon of stress. The lymphocytes exhibit "increased cytoplasmic-nuclear ratio, poorly basophilic cytoplasm, cellular distortion" (Frank et al., 1953) and vacuolation, perhaps as a prelude to cell death. This stress phenomenon is induced by epinephrine, histamine, and adrenocortical secretions (Frank et al., 1953). Furthermore, these authors state that cytoplasmic budding, pyknosis, and karyorrhexis are specific to adrenocortical secretions. Ingram (1960) concurs that pyknosis is induced by large doses of cortisone.

The appearance of blast or premitotic lymphocytes is usually an observational phenomenon; the data in Reference-Table II indicate that the number of blasts cells obtained with increasing dose is not significantly different, although a general increase to 100 R is noted. This parameter is further complicated by the response of lymphocytes to various antigenic stimuli (Robbins, 1960). It is impossible to rule out completely any antigenic stimulus to the lymphocytes in culture because this culture method in itself may provide a stimulus to the cells. The accident case which has been investigated here shows a departure from the experimental data in that a much larger percentage of blast cells was observed, then expected on the basis of Weijer's (1969) data. This phenomenon is probably due to the length of time elapsed between accidental irradiation and blood sampling. If there is an immunological stimulus imparted by irradiation, the time elasped in this case would certainly allow

for removal of the cells to some organ, for transformation of some of these cells, and for subsequent release back into the circulating blood.

In the cellular aberration method, the most reliable parameters are a) incidence of pyknotic cells, b) surviving lymphocytes, and c) surviving neutrophils. The response of these three cell types to increasing radiation doses is predictable with significantly different yields at each radiation dose, in the experimental data of Weijer (1969). The neutrophils are more resistant than lymphocytes to ionizing radiations, manifesting a higher percentage survival for doses up to 50R, in 72-hour cultures. This phenomenon is dependent on time in culture, since in preparations harvested from 84 and 96-hour cultures, the incidence of living neutrophils is low for all dose levels.

With respect to all these parameters, but particularly to the three standards just described, the time elapsed between irradiation and blood sampling becomes the limiting factor in dose estimation. The assumed linear relation between decay of assessable biological damage and time post-irradiation probably does not hold. Jacobson, (1950), Mathe, (1964), and Dienstbier et al.,(1966), have shown that maximum depression in leukocyte counts occurred between the first and third day post-irradiation; and that the lymphocyte count fluctuates for two days to seven weeks (depending on dose) before returning to normal. For the decay curve, the assessment at six to seven weeks represents a time when the damaged cells are being gradually removed from the blood. The 'fast decay' component for the period one-to-three days post-irradiation may be represented tentatively by the

slope of the depression curves (Fig. 5b, 5c; Appendix I) (for the same period) of Mathé and Bernard, (1960) and Mathé, (1964) since these curves represent removal of the biologically damaged cells and therefore a decay in assessable biological damage. The decay curve envisioned by Weijer is represented in Figure 5a (Appendix I).

At seven weeks post-irradiation the level of the lymphocytes has probably returned to a normal value and the blood counts carried out at three intervals may represent a normal variation in the lymphocyte count; at least, these fluctuations cannot be likened to the immediate radiation depression of Mathé.

It can be assumed that the victim of the accident has been exposed to a fractionated dose of ionizing radiation. The film dosimeters registered two doses of which the second was twice as high as the first. Since there are no data available on the effect of a fractionated dose on surviving lymphocyte frequencies, no correction for the dose estimate can be advanced. Furthermore, no correction has been made for secondary effects due to the time lapse. These include recovery in vivo of cells that would have become pyknotic if cultured immediately post-irradiation, and, if this was a bone marrow dose, the resulting disruption of normal cell production. For this case, then, the cellular aberration method of dose estimation can be used with limited success. However, the method certainly does corroborate an irradiation exposure and establishes a lower limit to the estimate of exposure dose.

Aberrant Chromosome Morphology

According to the work of Bender and Prescott (1962), the rate

of mitosis in peripheral blood lymphocytes (*in vivo*) is very low, and in culture the cells pass out of the G_1 phase only when antigenically stimulated. Irradiation of the blood *in vivo* should produce pre-replication chromosome breaks when these cells are cultured and stimulated to divide. Providing the lifespan of the lymphocyte is long, the induced aberrations should be preserved *in vivo*, and culturing these at some time after the irradiation should yield almost the same number of aberrations as culturing immediately post-irradiation. In practice this is not entirely true. Normal death of lymphocytes causes some decline in the number of cells with aberrations, and aberrations within the cells will accelerate the ageing process. Buckton *et al.*, in several publications, have shown a decline in the number of cells with unstable and stable aberrations. Part of this is due to normal death, and part can be ascribed to biochemical changes in the cells, due to the rearranged chromosomes, which shorten their survival time. In the case described here, some of the cells which appear pyknotic at seven weeks post-irradiation might have shown severe nuclear aberrations at three days post-irradiation. This may partially account for the small number of dicentric and ring chromosomes observed in these cultures.

The number of hours in culture also has a bearing on the preservation of aberrations. Buckton and Pike (1964, 1964), Sasaki and Norman (1966) and Norman (1967) have demonstrated that the first wave of mitotic metaphases can be harvested at 52 hours in culture, and that the majority of cells in metaphase at 72 hours in culture are in their second division post-irradiation. Most of these 72-hour

metaphases will not show unstable aberrations since dicentrics, rings, and fragments are usually lost at the first division post-irradiation.

The large number of chromatid aberrations observed is not expected in the analysis of peripheral blood lymphocytes irradiated in vivo. The presence of this type of aberration indicates irradiation of the actively dividing stem cells in the bone marrow. Whether the kinetics of aberration production in the stem cells are of the same order as those of cultured cells is not known for human exposure. However, the yield of aberrations in this accident case could not be correlated with dose purely on the basis of chromosome-type aberration kinetics as expressed by the Bender and Gooch (1962) formula, because of the large contribution of chromatid aberrations and the attendant differences in coefficients of production of this type of aberration. Analysis using both systems yields almost identical dose estimations, which are close to the dose registered on the film badge dosimeter.

The physical dose of irradiation in an accident case does not necessarily represent the effective biological dose. Usually it is assumed that the accidental exposure involved the total body, whereas in reality the dose may have been confined to a part of the body. The biological dose must be interpreted in terms of gonadal involvement, since a total body exposure exclusive of the gonads is not potentially harmful to another generation. For total body doses below the LD_{50}, the body can repair most of the damage done by ionizing radiation by replacement of the damaged tissue. For very low doses the damage is

minimal. However, even very low doses of ionizing radiation can cause irreversible changes in the chromosomes of gametes. In this accident case we can neither confirm nor negate a possibility that the physical dose represents this effective biological dose. There is no adequate method at present for obtaining and culturing human gonadal cells, nor is there data available on which to base any conclusions regarding the gonadal dose.

Chromosome Associations

"Stickiness" is observed in chromosomes after irradiation at high doses, but this is usually manifested as a generalized clumping of the chromosomes. The specific chromosomes associated in this case suggest a genetic abnormality which seemingly has not produced any outward manifestation. These associations are in the same groups as noted by Ferguson-Smith and Handmaker (1963): "The chromosomes most obviously associated with one another are the satellited chromosomes (numbers 13, 14, 15, 21, 22) which are often seen linked together by their short arms, the phenomenon of satellite association." and further: " It appears that the feature common to all significant chromosome associations is that they tend to occur close to sites where secondary constrictions have been demonstrated." (p. 143).

The hypothesis presented by these authors to explain this phenomenon involves a fusion of the nucleoli and consequent association of the nucleolus-organizers of each chromosome, the site of which is near a secondary constriction on the telocentric chromosomes; namely, one or more of the 13-15 or 21-22 groups. When the nucleolus disappears prior to division, the association remains, persistent throughout

late metaphase. Thus this association is not necessarily a genetic abnormality since it is reported to occur commonly without any outward manifestations. This is illustrated by the following data, compiled from investigations carried out by Barbara Chernick (personal communication):

Blood Donor	Total Number of Metaphases	Number of Metaphases with at least one of the following Associations: D-D, D-G or G-G	%
BC	29	21	72.4
BC	18	14	77.8
BC	18	13	72.2
BC	9	7	77.8
BC	24	18	75.0
BC	16	14	87.5
BC	8	6	75.0
BC	16	14	87.5
SU	38	32	84.2
JK	72	43	59.7
PM	24	12	50.0

The associated chromosomes appeared to be of normal length; it was necessary to cut through the association 'thread' for the karyotyping, but the association is evident on the inset photomicrograph (Fig. 16.17, Appendix I).

SUMMARY

The case of an accidental exposure to X-irradiation has been investigated using 'biological dosimeters' to confirm the dose indicated on the film badge dosimeter. The high numbers of aberrant cells and chromosomal aberrations, when interpreted on the basis of data accumulated by Weijer and Weijer (1969), and by Bender *et al.* (various publications), establish a minimum dose of exposure of 60R and a probable maximum of 102R. On the basis of a high frequency of chromatid-type breaks it is assumed that this dose was also received by the bone marrow.

The chromosome associations observed are not due to radiation-induced stickiness, but probably represent a genetic abnormality which has not been outwardly expressed.

APPENDIX I

REFERENCE-TABLE I

Coefficients of Chromosomal Aberration Production for Human Leukocytes *in vitro*

Chromatid Aberrations		Chromosome Aberrations		Reference
Deletions/cell per R	Isochromatid deletions/ cell per R	Deletions/ cell per R	Rings and dicentrics/ cell per R^2	
0.39×10^{-2}				Bell & Baker, 1962
0.24×10^{-2}		0.11×10^{-2}	0.45×10^{-5} 0.52×10^{-5} 0.70×10^{-5}	Bender & Gooch, 1962b
0.26×10^{-2}				Bender & Gooch, 1962c
0.149×10^{-2}	0.150×10^{-2}			Bender & Gooch, 1963
		0.90×10^{-3}	6.0×10^{-6}	Bender & Gooch, 1968
			2.7×10^{-6}	Norman *et al.*, 1964
			5.7×10^{-6} (50 hr.) 3.1×10^{-6} (72 hr.)	Norman, 1967

REFERENCE-TABLE II

Cell Survival and Cellular Aberration Production in Human Leukocytes *in vitro*

Dose, R	Pyknotics	Surviving Lymphocytes	Neutrophils	Blasts	Binucleates
0	10.71±2.38	89.52±4.37	1.25±0.87	1.84±1.25	1.25±0.84
25	19.60±2.59	79.30±2.76	4.34±1.35	2.75±1.53	2.89±1.67
50	19.36±2.56	73.68±2.28	9.31±1.47	4.04±1.50	3.57±1.61
100	34.23±3.10	66.16±2.36	1.72±0.83	6.01±1.99	4.95±1.55
200	66.02±4.61	34.16±1.91	1.07±1.11	4.55±1.13	5.36±1.61
300	68.34±5.12	14.10±2.51	0.76±0.67	1.54±0.82	4.35±1.58
400	71.13±4.94	3.35±2.46	0.45±0.50	0.58±0.70	2.74±1.50

(Weijer & Weijer, 1969)

Figures 1-4: Dose-effect relationships from the experimental data of Weijer (1969)

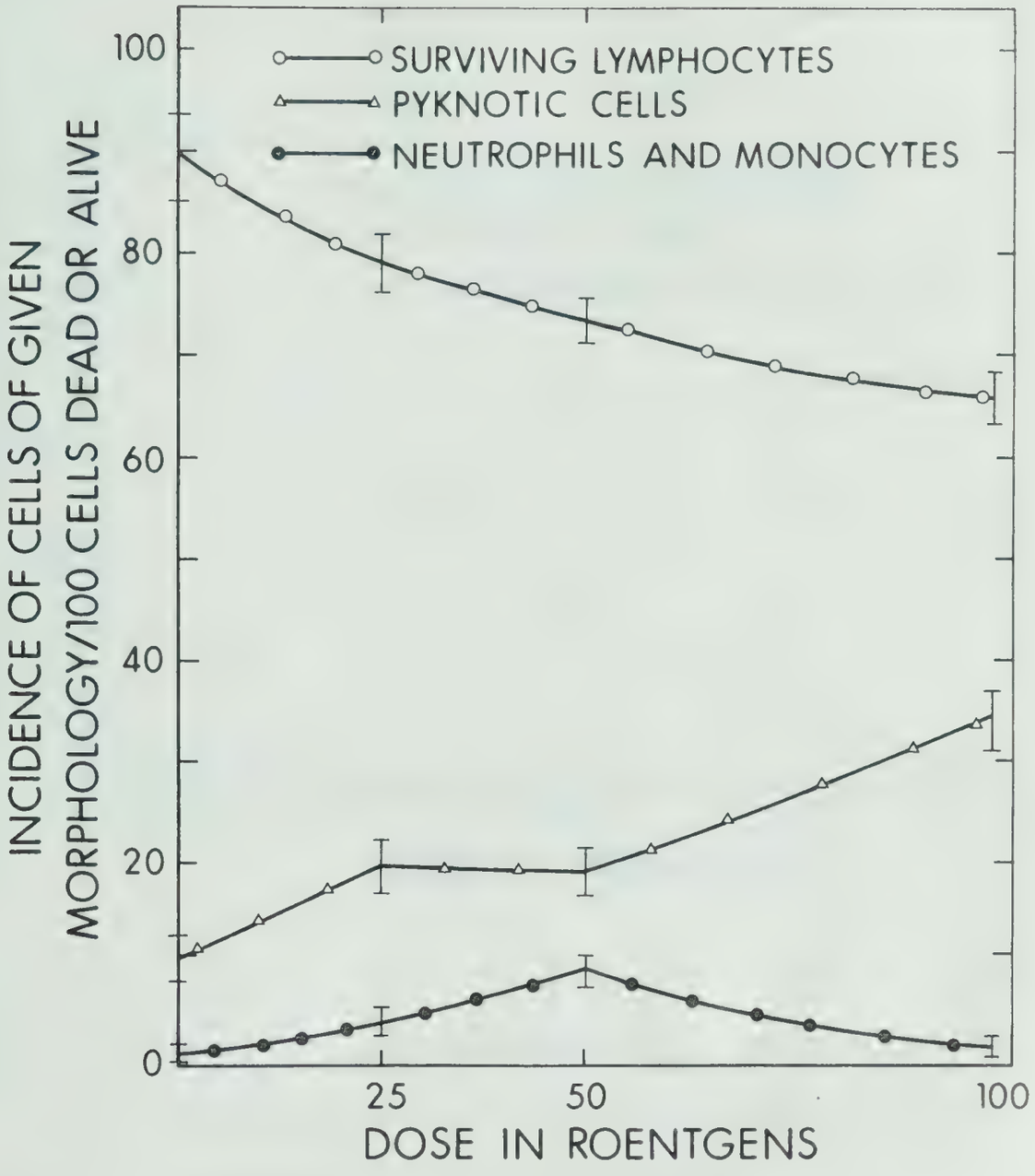


Fig. 1. Effect of increasing single doses of X-rays, 0-100R, on incidence of surviving and pyknotic cells in culture (72 hr. post-irradiation).

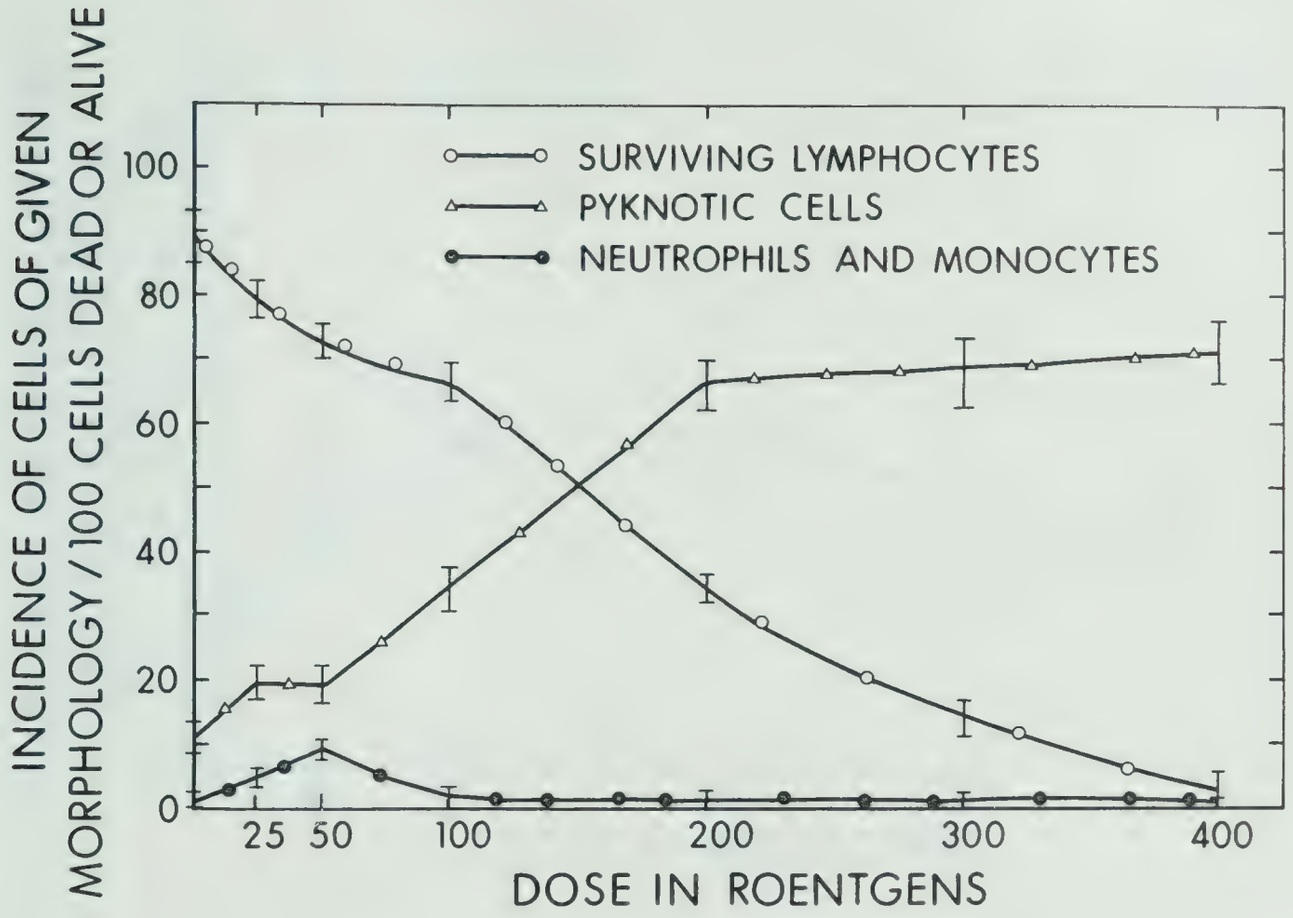


Fig. 2. Effect of increasing single doses of X-rays, 0-400R, on incidence of surviving and pyknotic cells in culture (72 hr. post-irradiation).

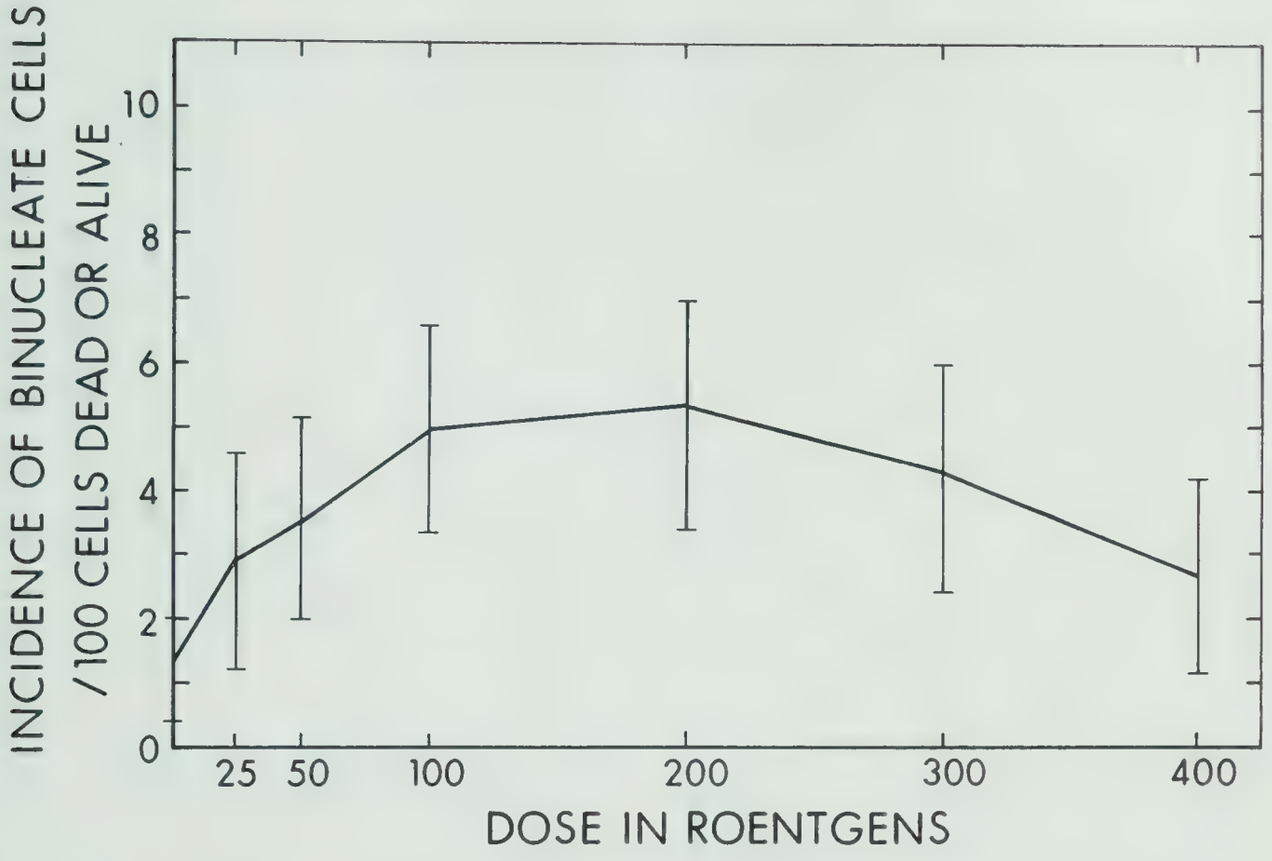


Fig. 3. Effect of increasing single doses of X-rays, 0-400R, on incidence of binucleate cells in culture (72 hr. post-irradiation).

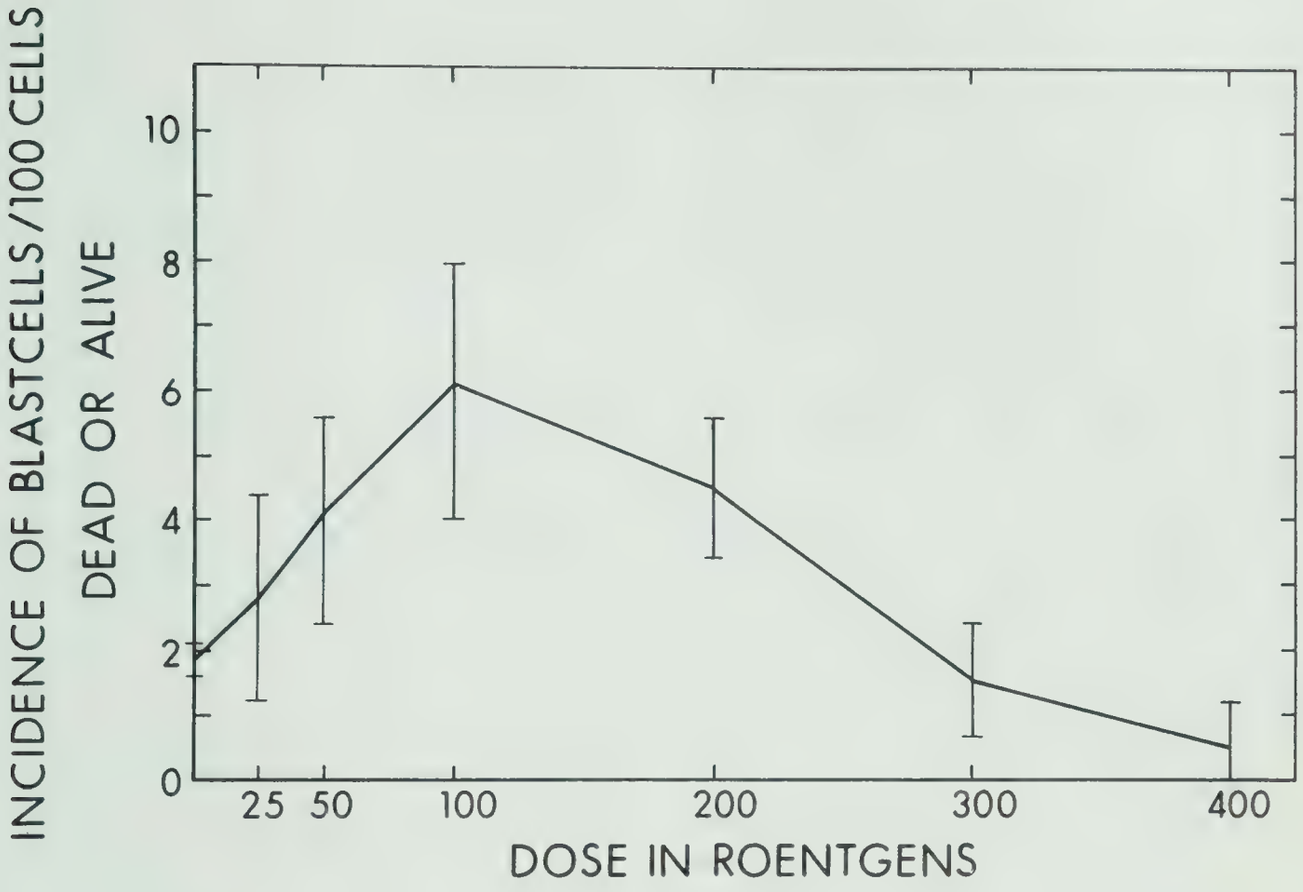


Figure 4. Effect of increasing single doses of X-rays, 0-400R, on incidence of blast cells in culture (72 hr. post-irradiation).

Figure 5. Lymphocyte depression after *in vivo* irradiation and assessment of biological damage.

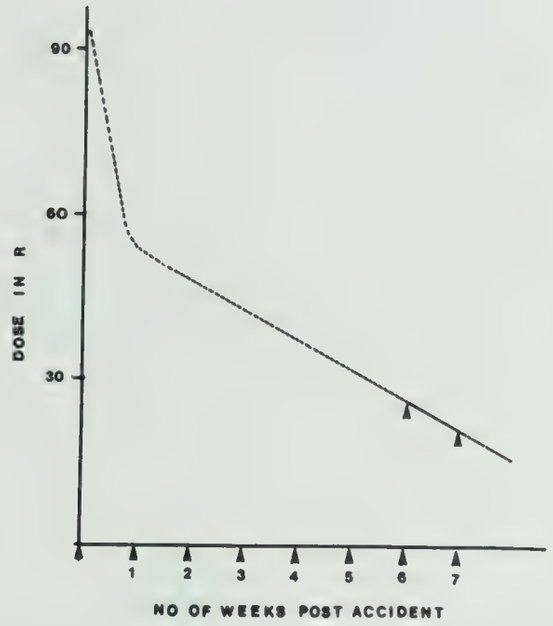


Figure 5 a. The relationship between assessable dose (in R) and time post-irradiation (in weeks). (Weijer and Weijer 1968)

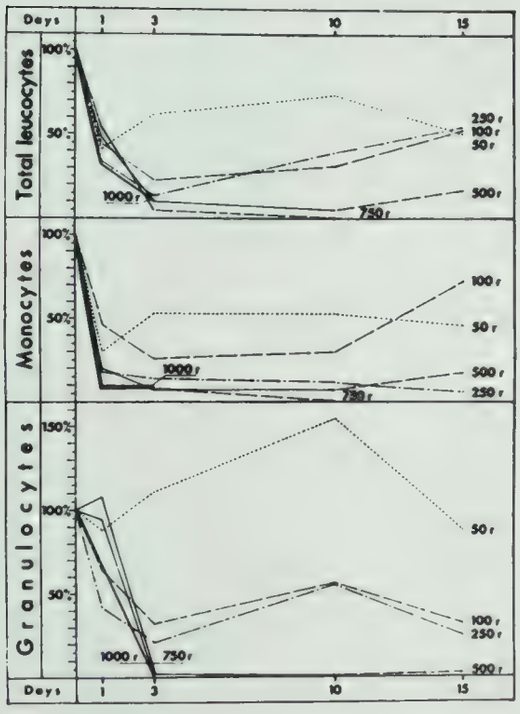


Figure 5 b. Post-irradiation leukocyte depression and recovery in mice. (Mathé and Bernard, 1960)

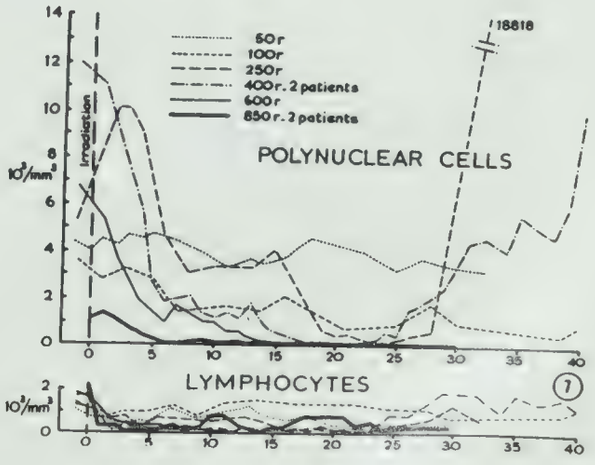


Figure 5 c. Post-irradiation leukocyte depression and recovery in man. (Mathé, 1964.

PLATES I - III

Photomicrographs of accidentally irradiated peripheral leukocytes, cultured *in vitro* for 72 hours. Normal and aberrant cellular morphology at six to seven weeks post-irradiation.

b = lymphocytic blast cell

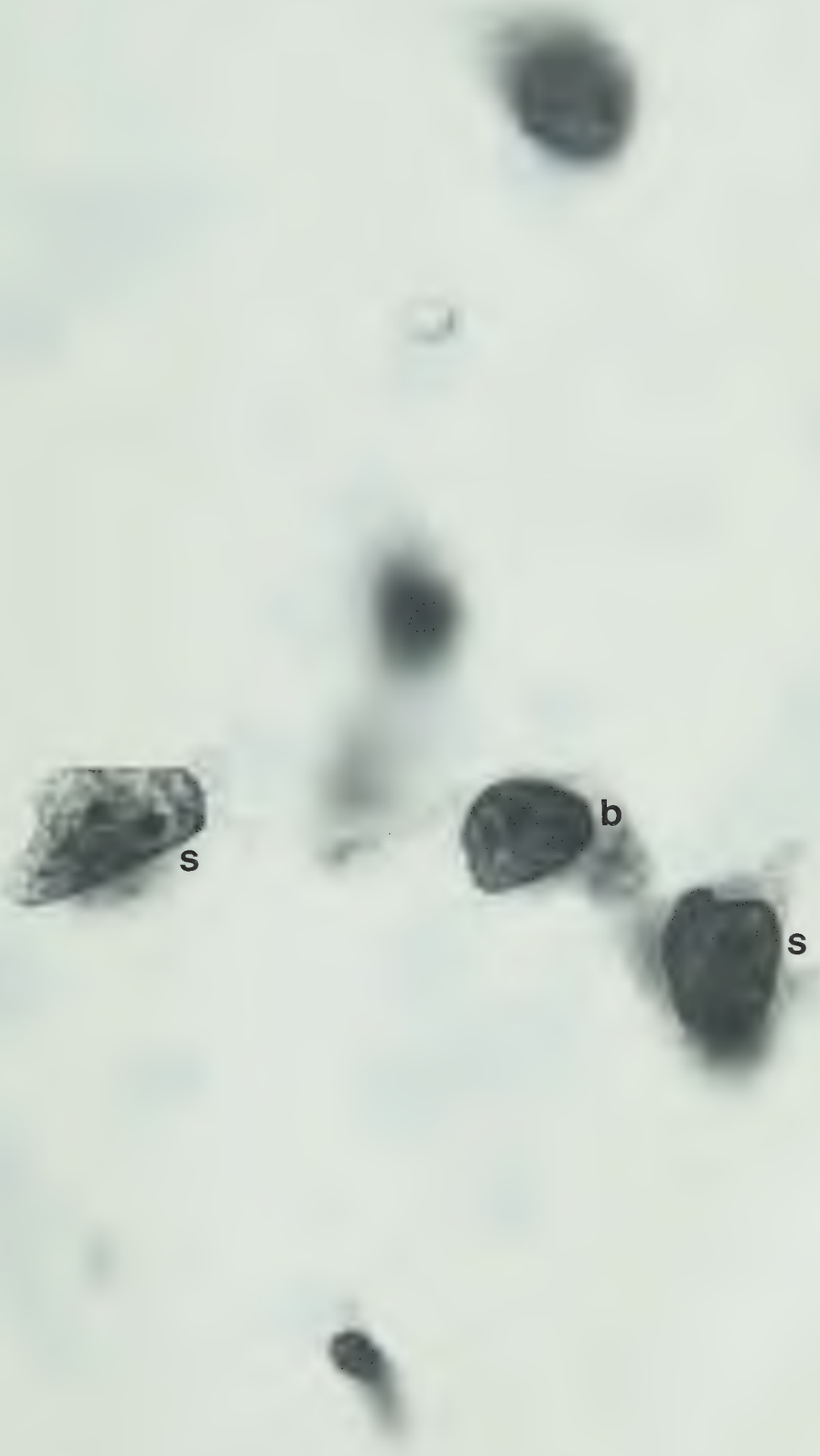
bin = binucleate cell

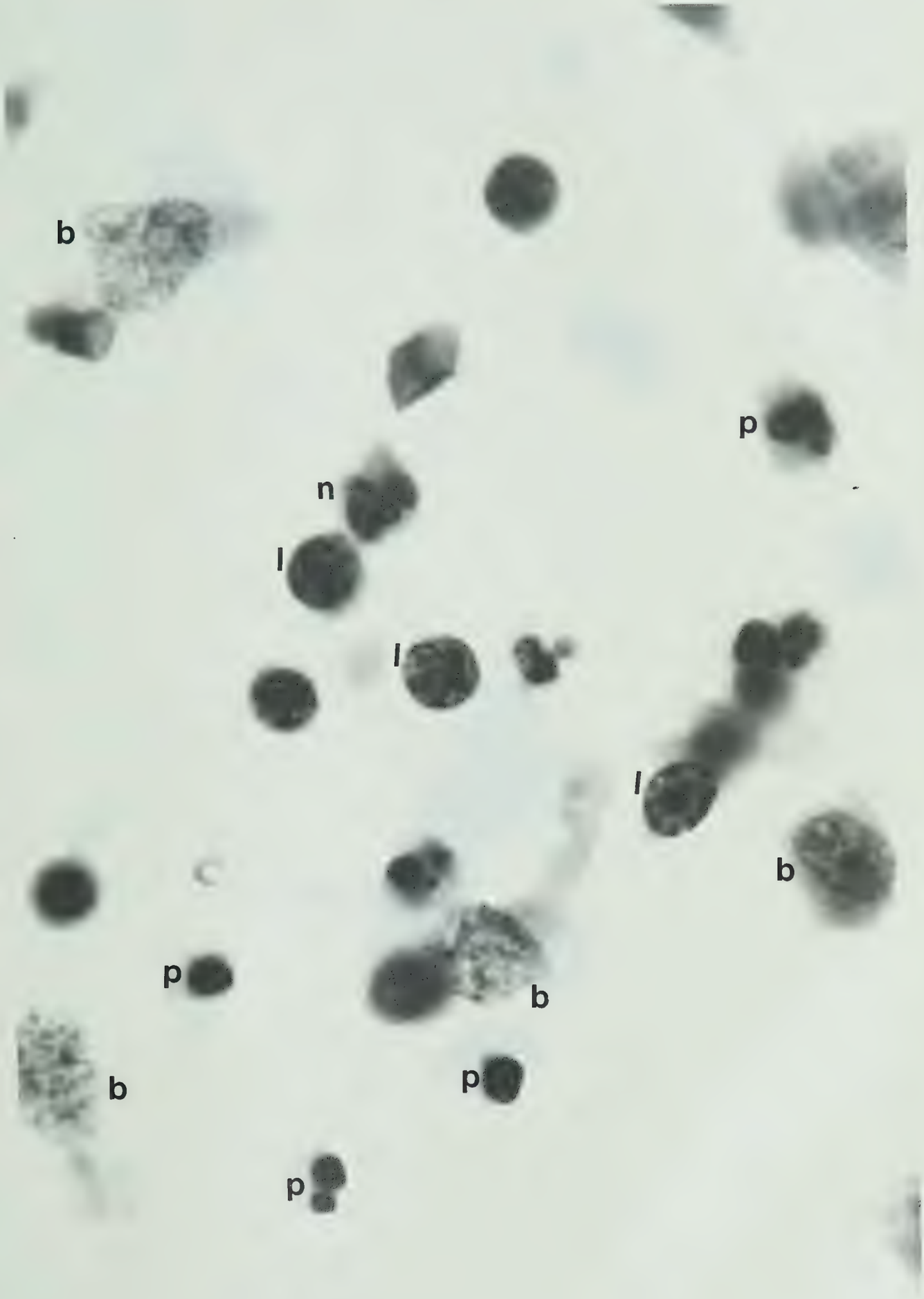
l = lymphocyte

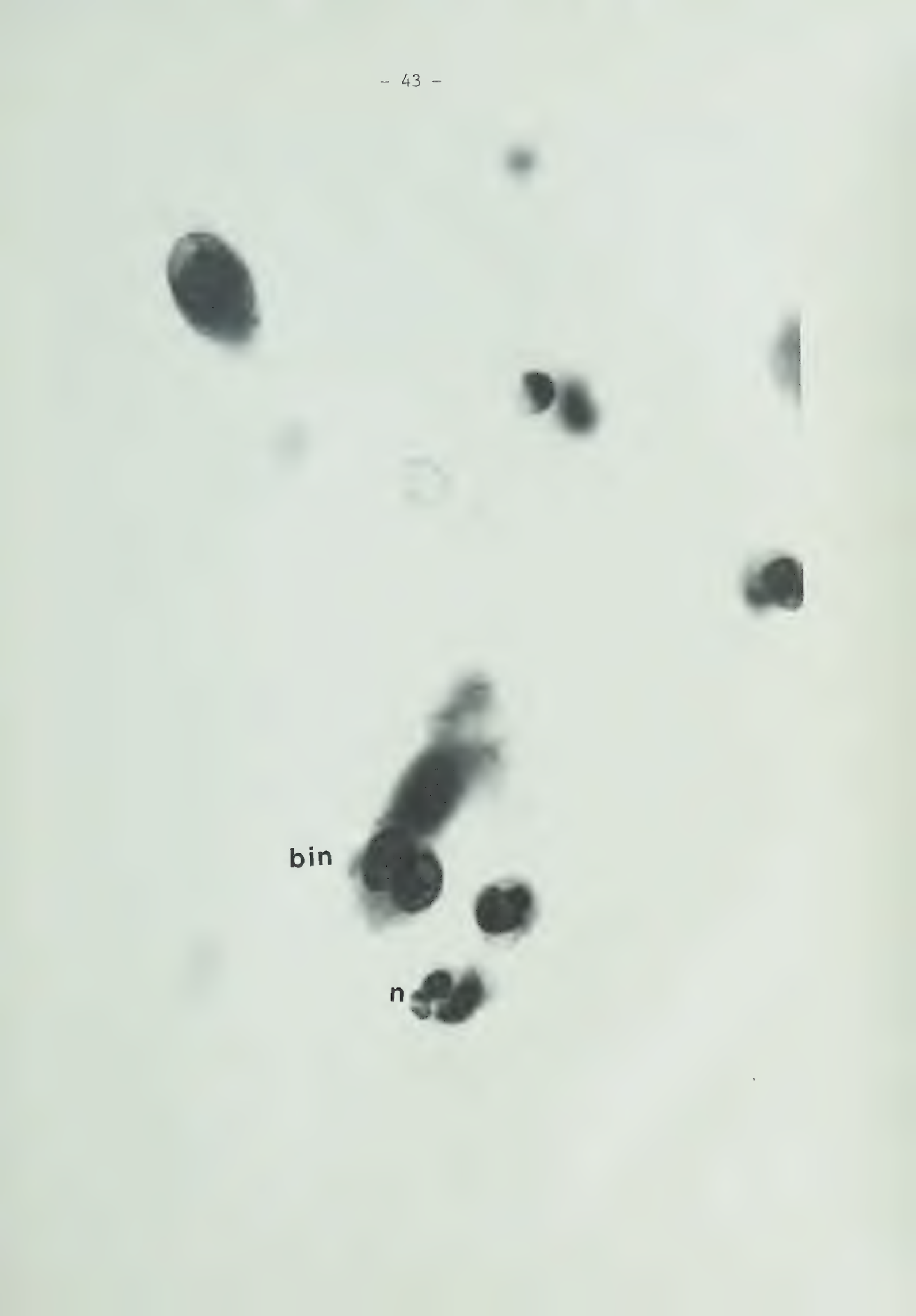
n = neutrophil

p = pyknotic cell

s = lymphocytic blast cell showing 'stress' phenomenon.


s
b
s


b
p
n
l
l
l
b
p
b
b
p
p


bin
n

PLATES IV - XV

Photomicrographs of accidentally irradiated peripheral leukocytes cultured *in vitro* for 72 hours. Metaphase plates exhibiting chromosomal aberrations and chromosome associations; six to seven weeks post-irradiation.

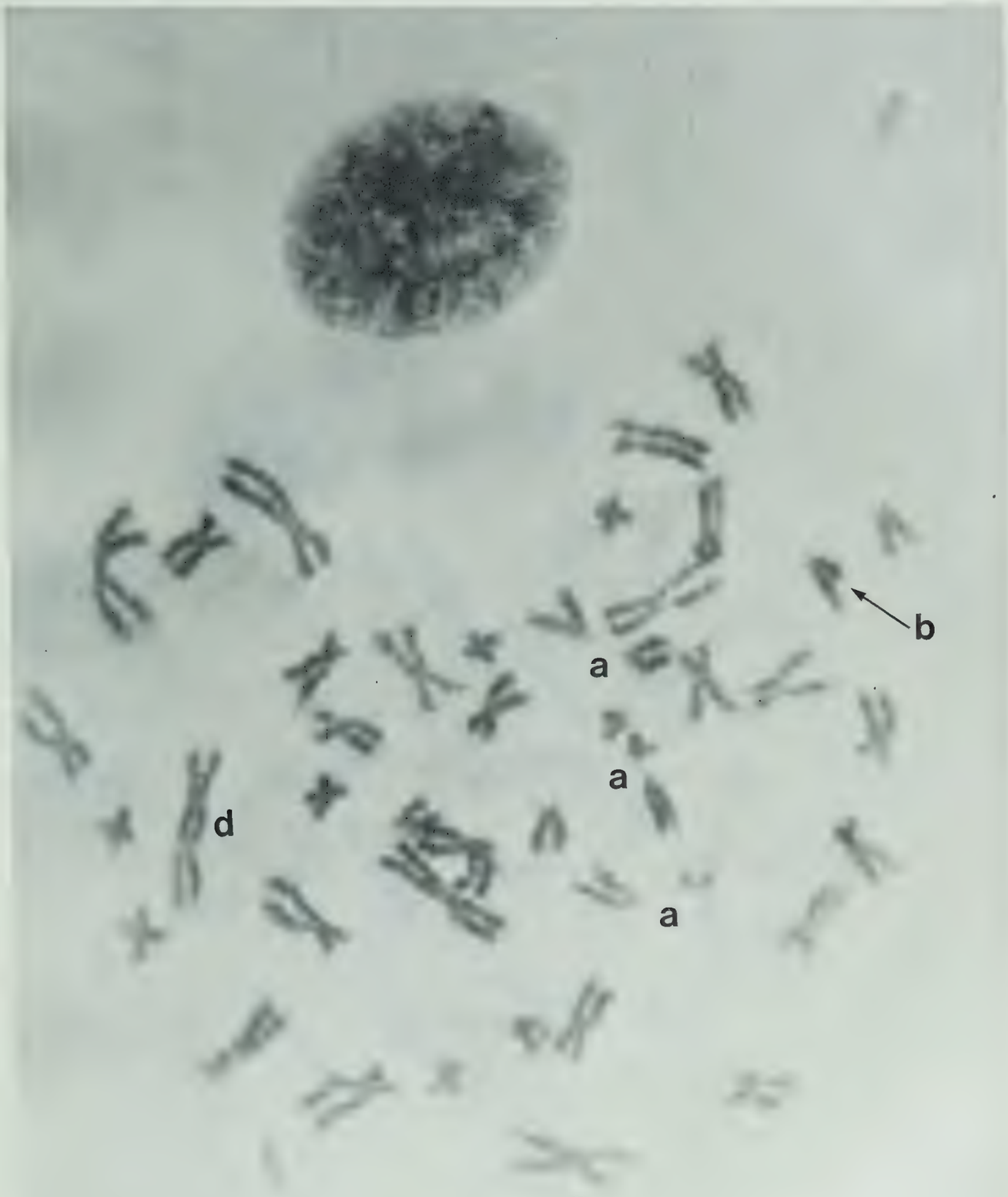
a = chromosome association

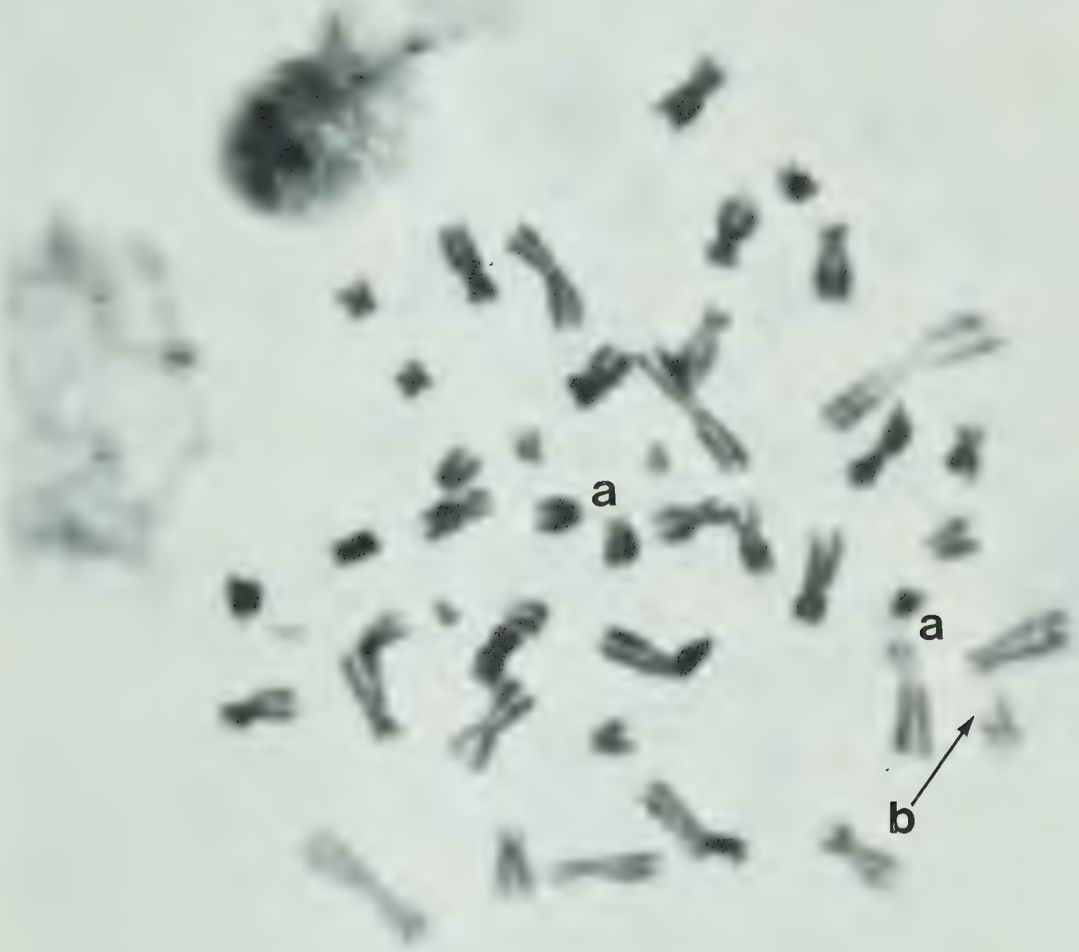
b = break (chromosome or chromatid)

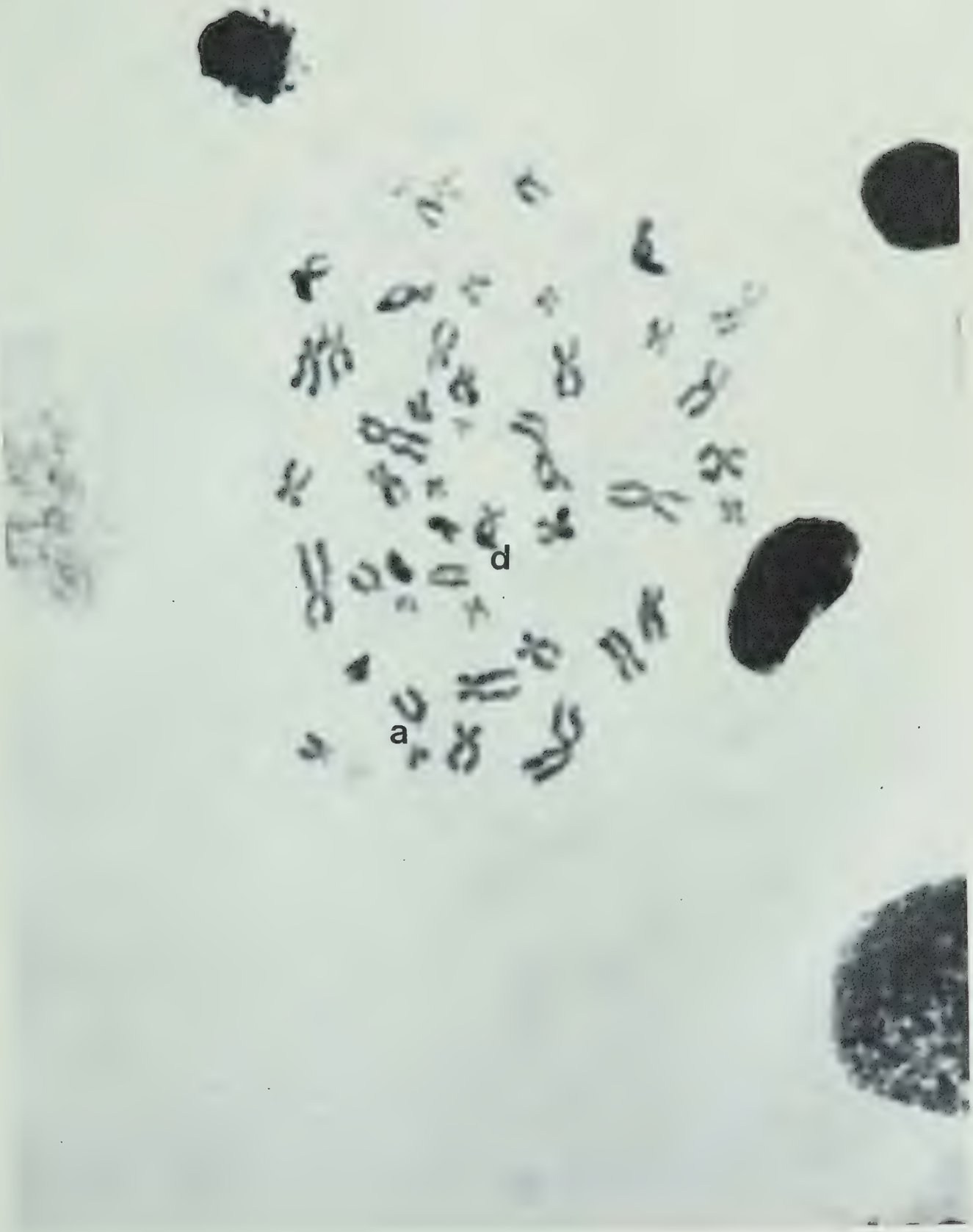
d = dicentric

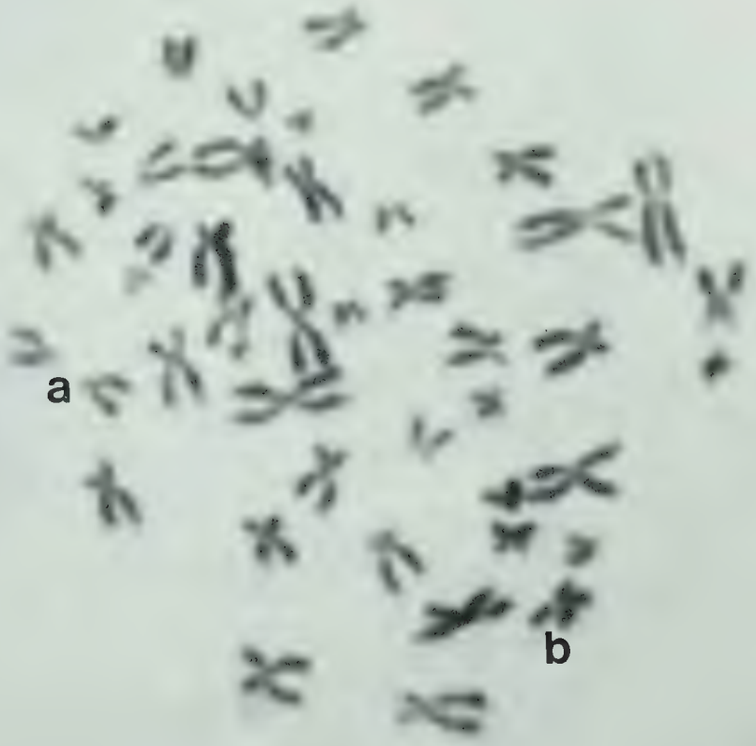
f = fragment

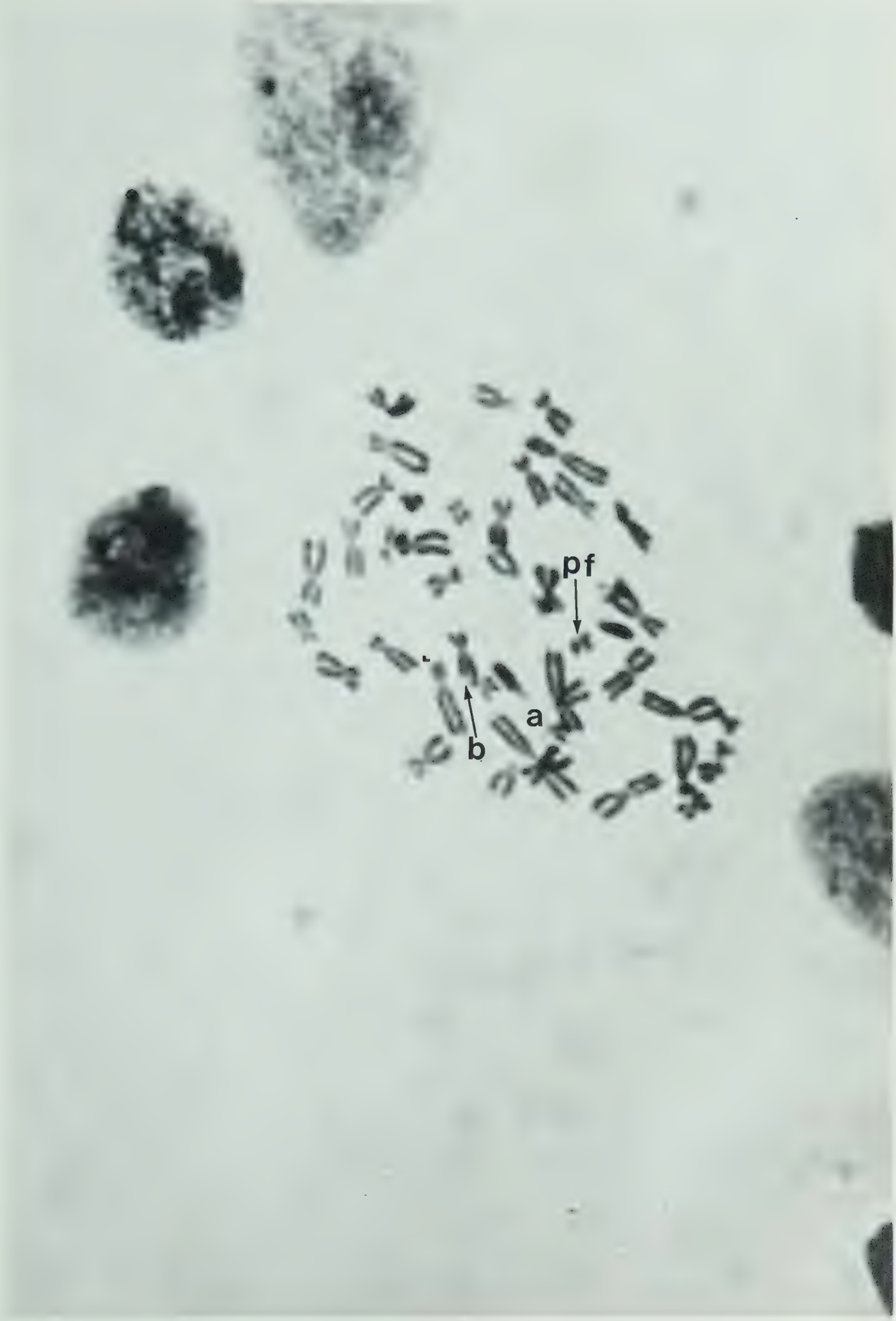
pf = paired fragment

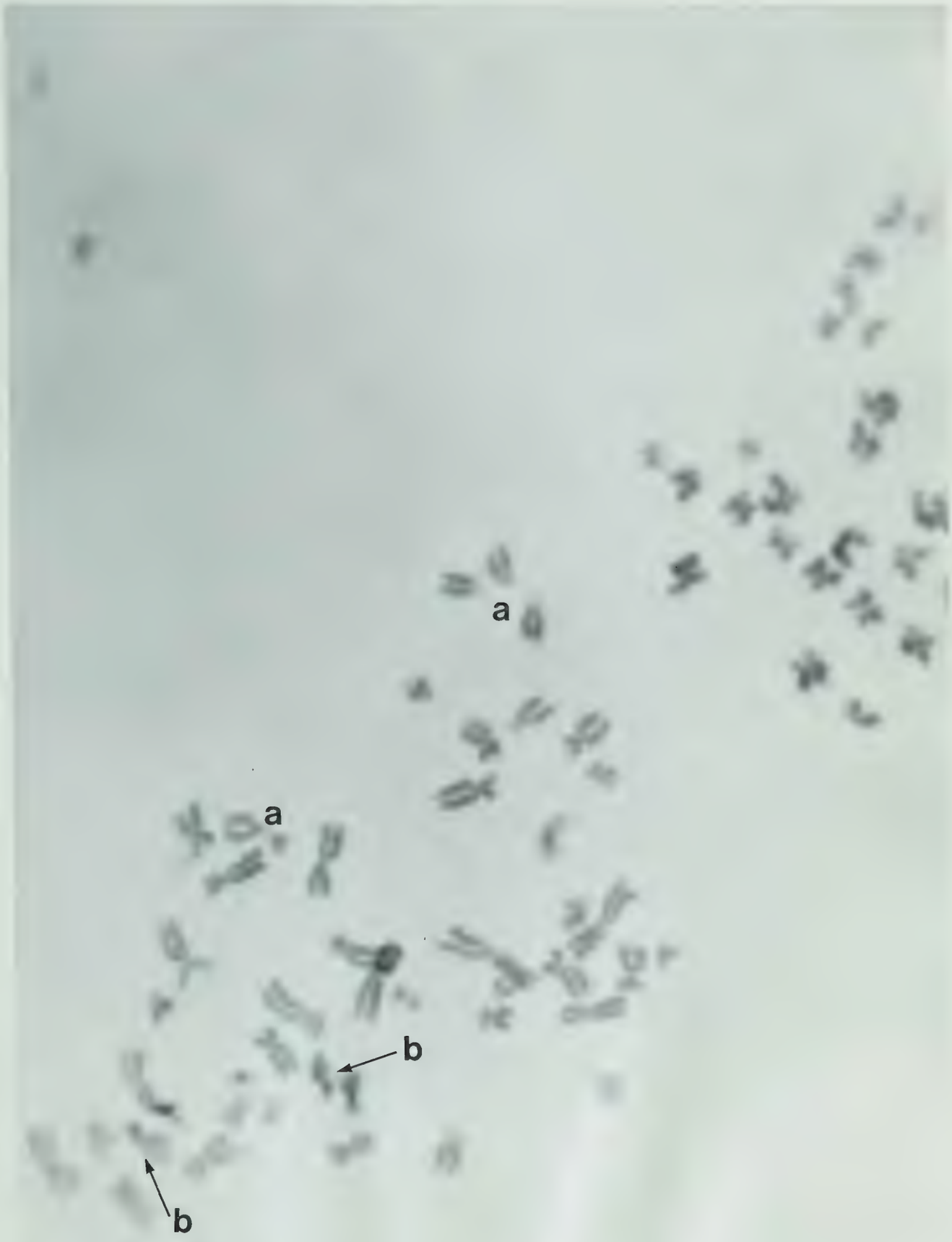

b
a
a
a
d

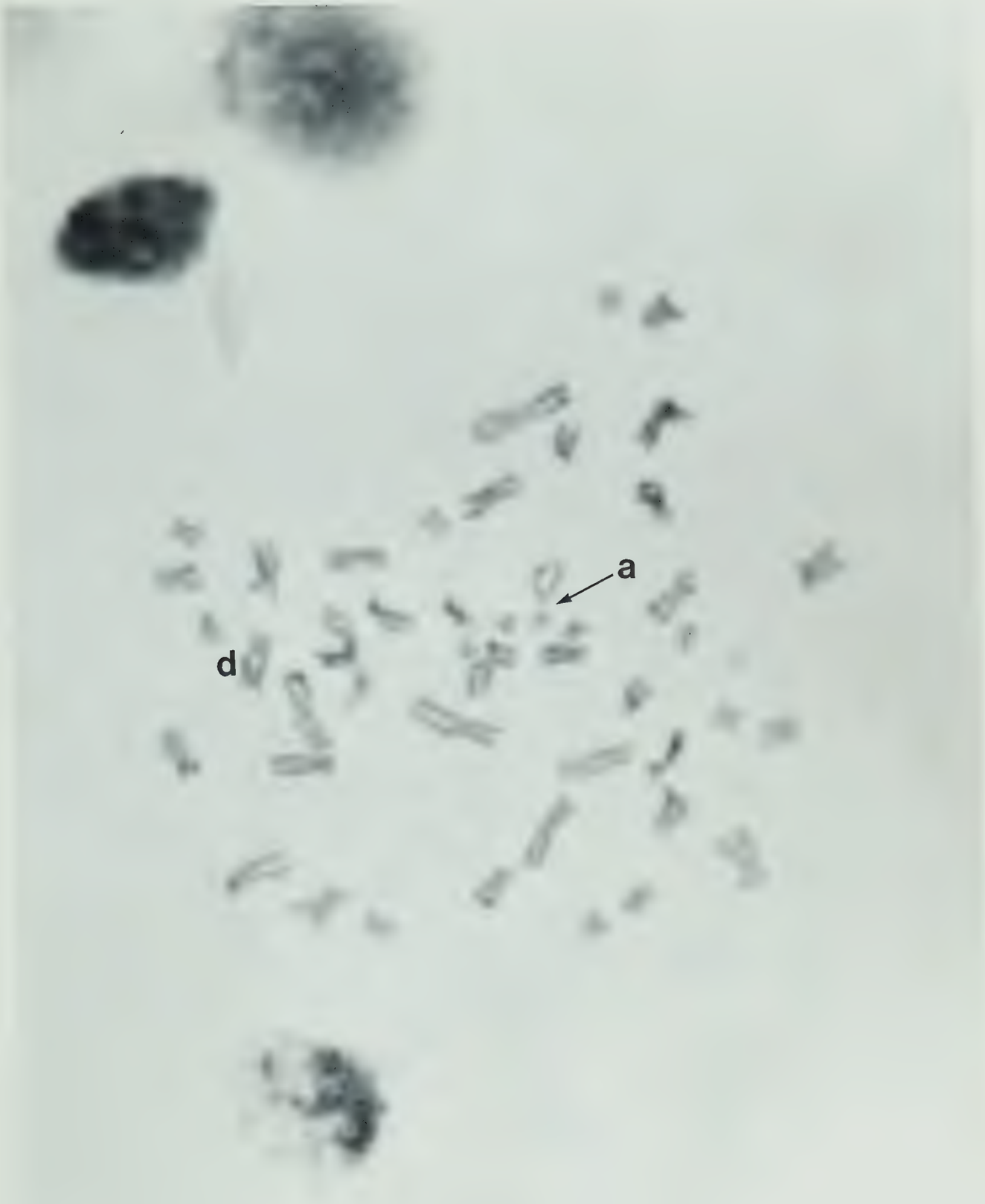

a
a
b

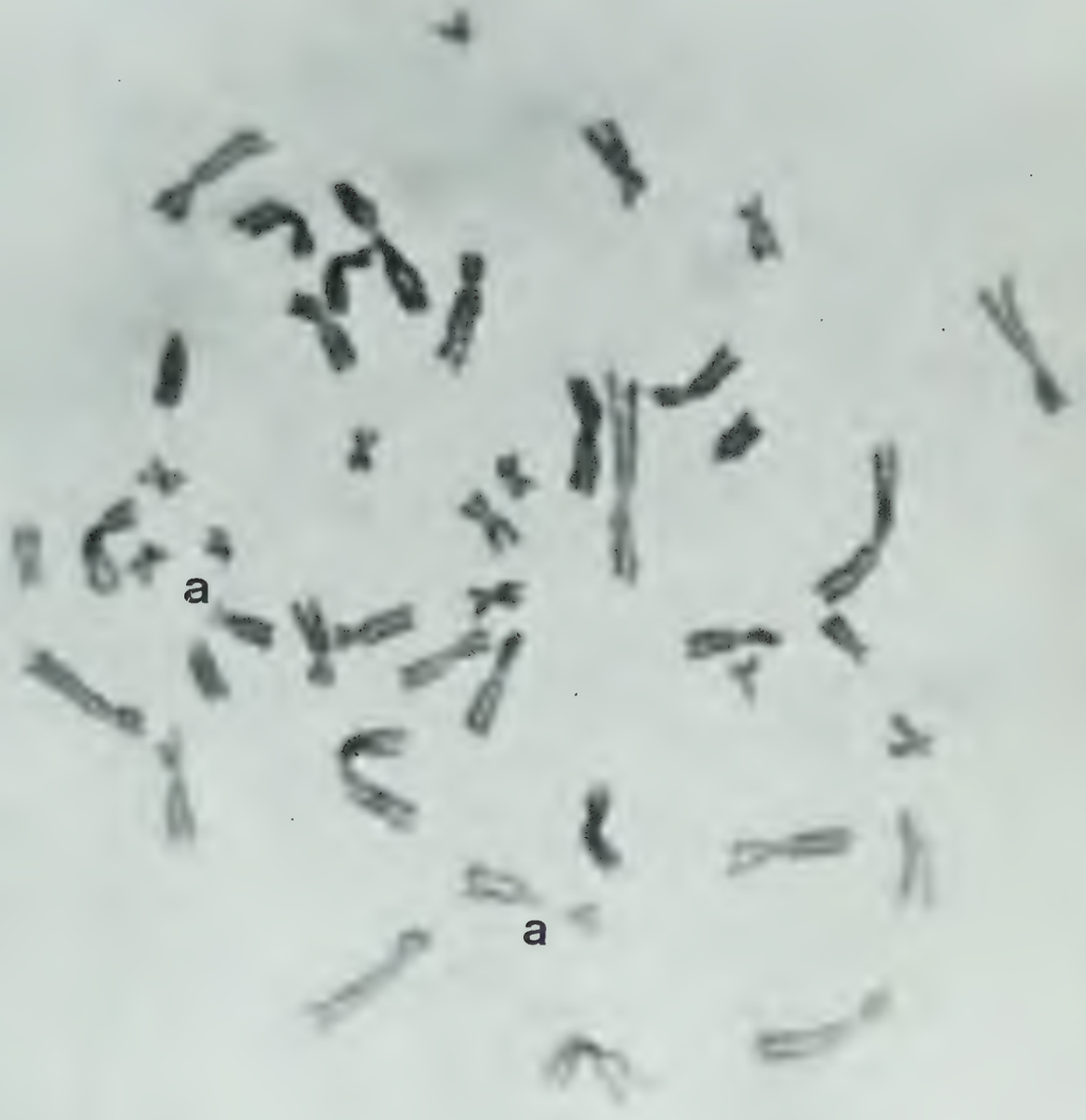

d
a

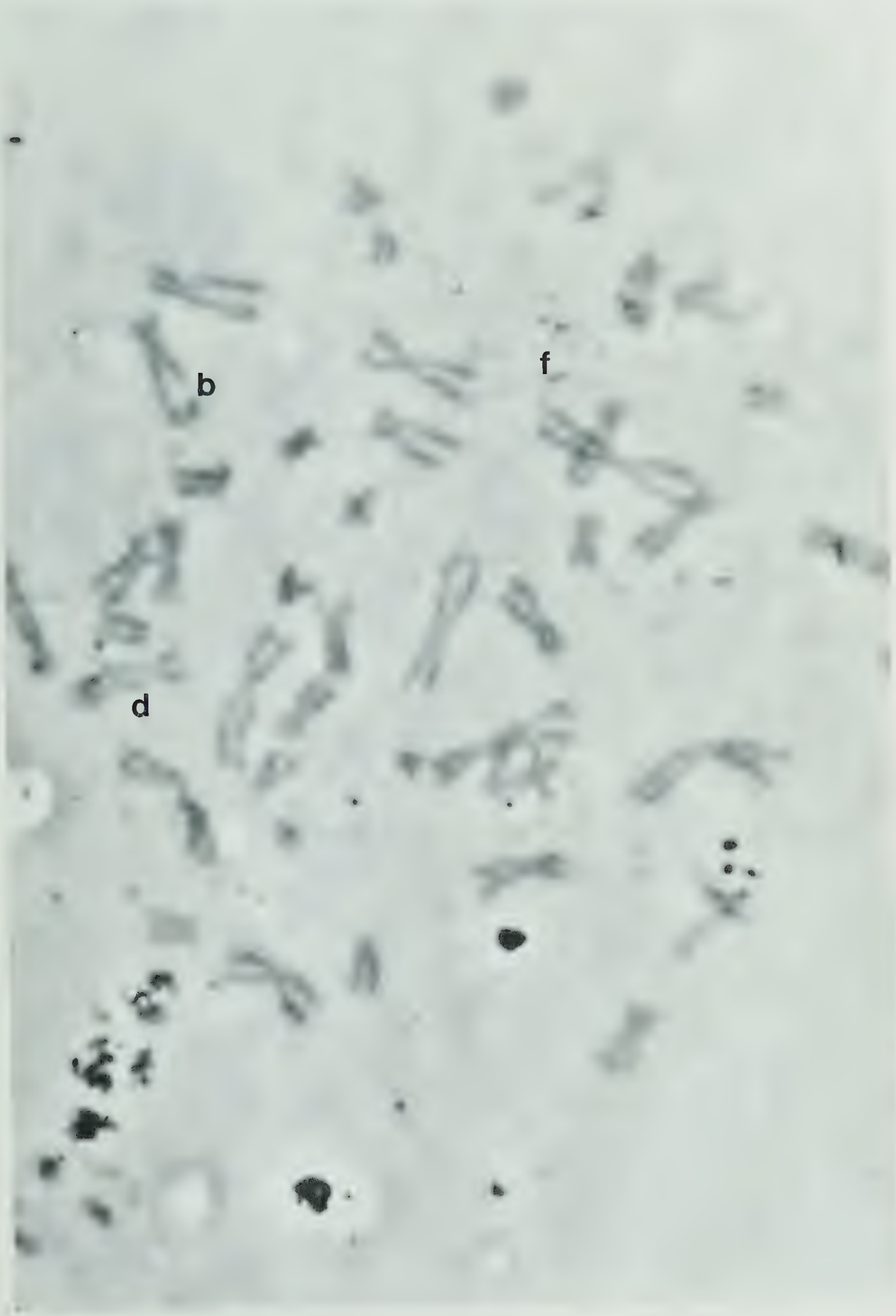

a
b

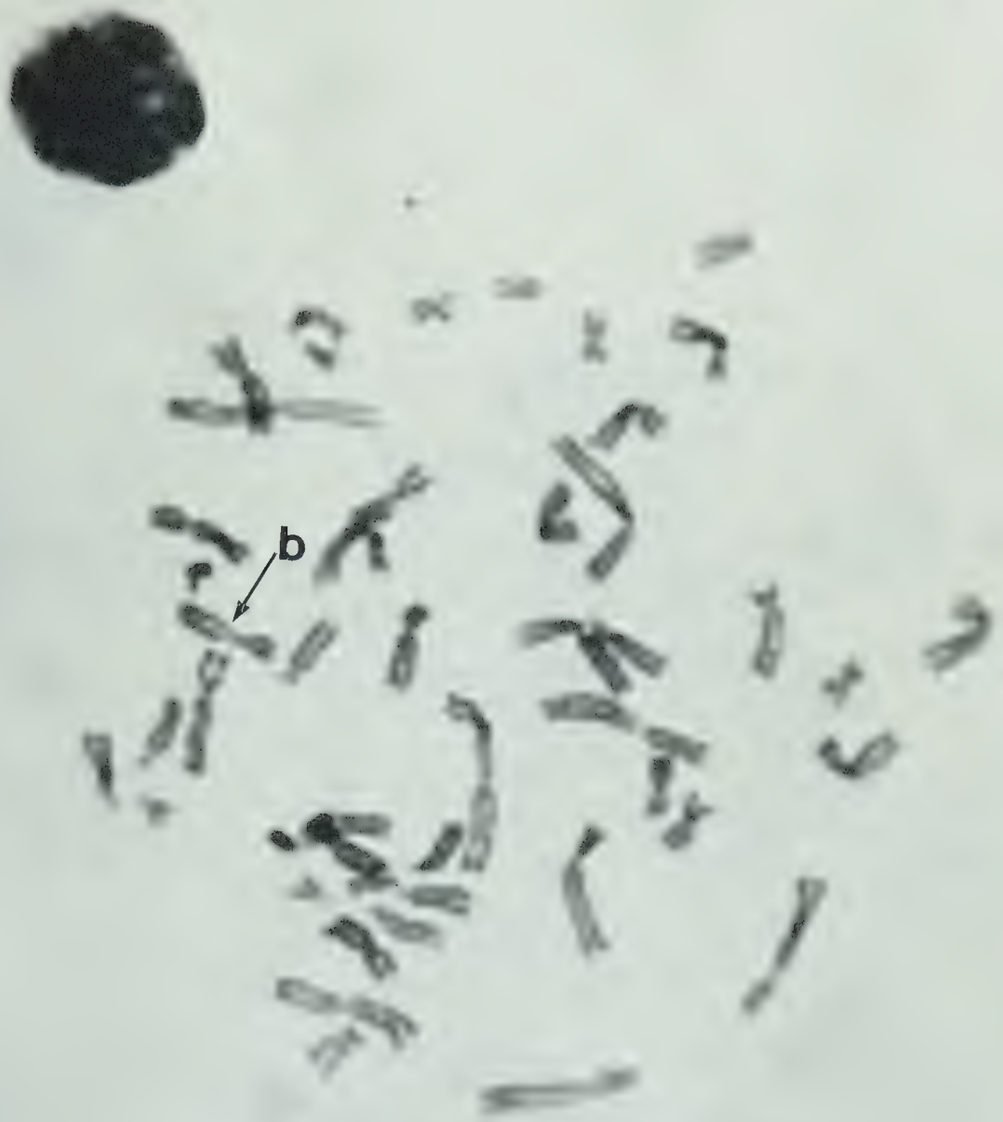

pf
a
b

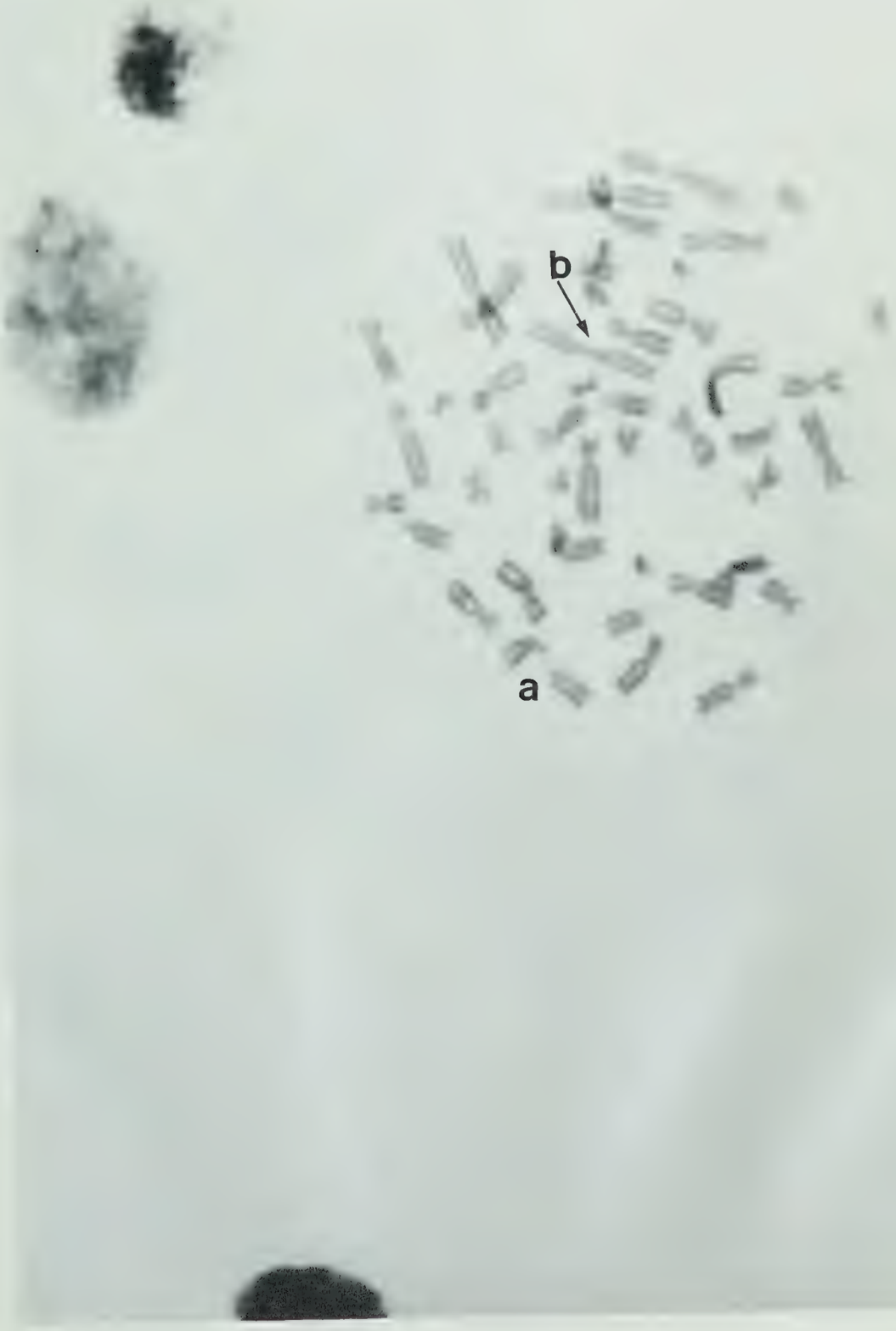

a
a
b
b

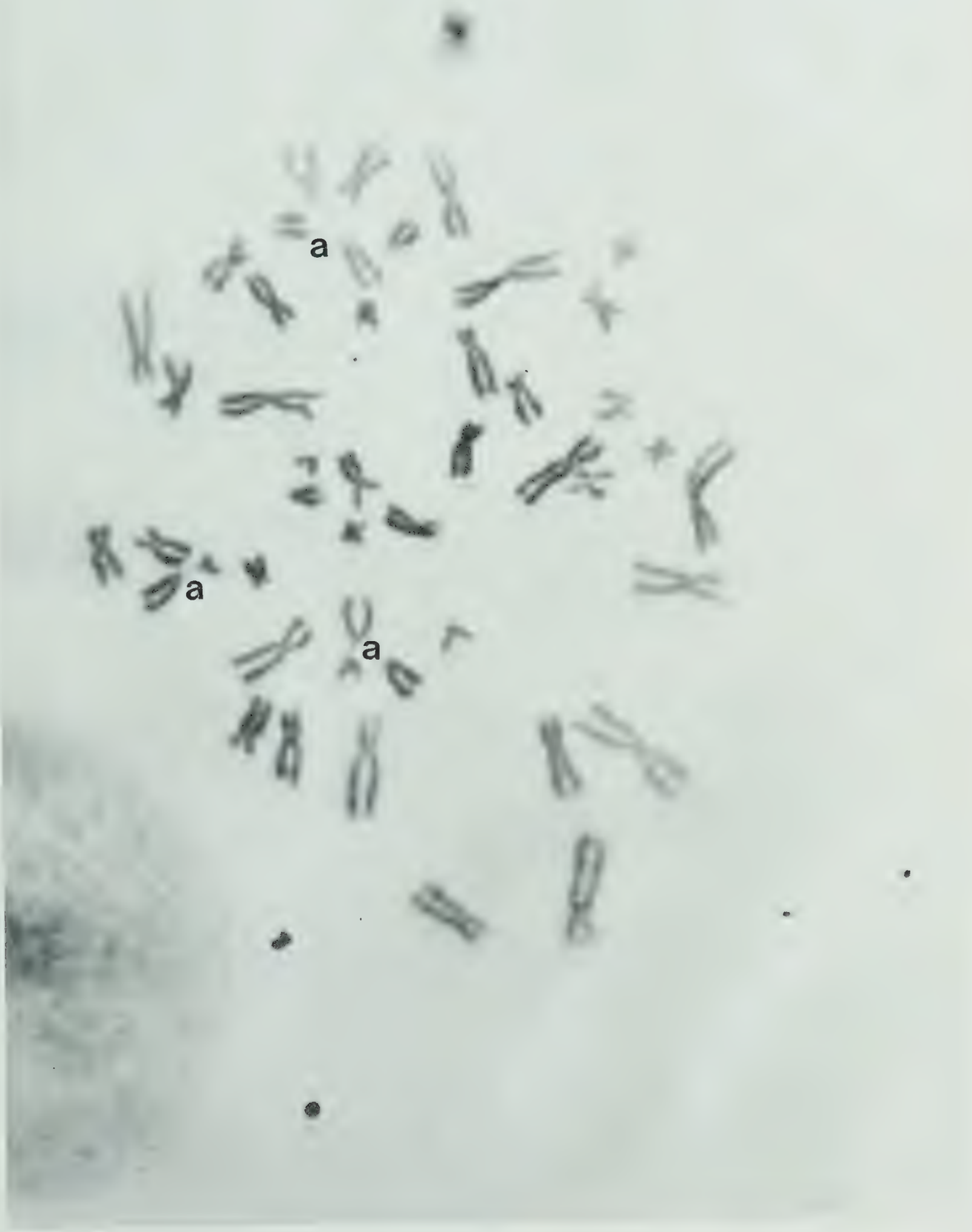

a
d


a
a


b
f
d


b

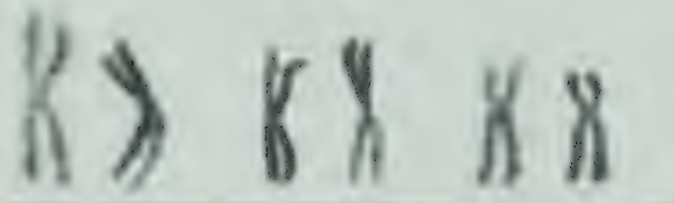

b
a


a
a
a

PLATES XVI - XVII

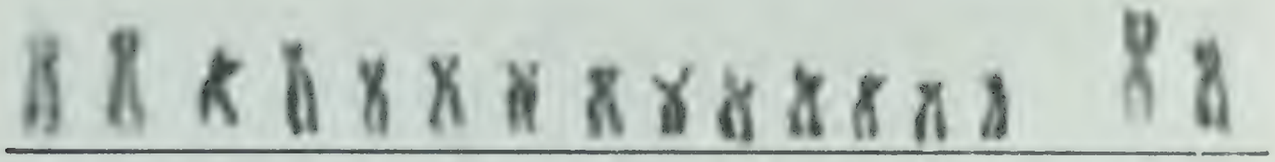
Karyotypes prepared from Plates XIV and XV, identifying chromosomes involved in associations.

X 1700

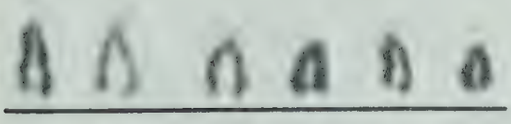


1 2 3

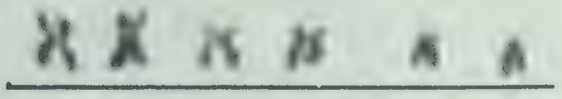
4 — 5



6 — 12 XX



13 — 15



16 — 18

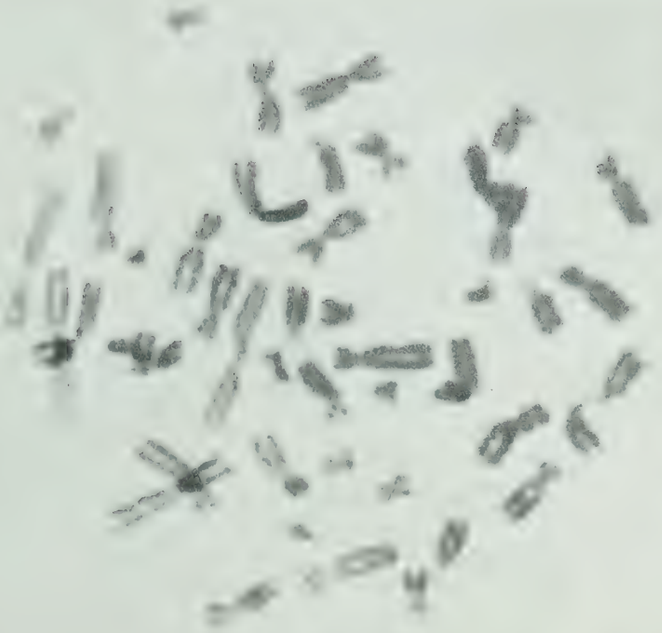
19 — 20

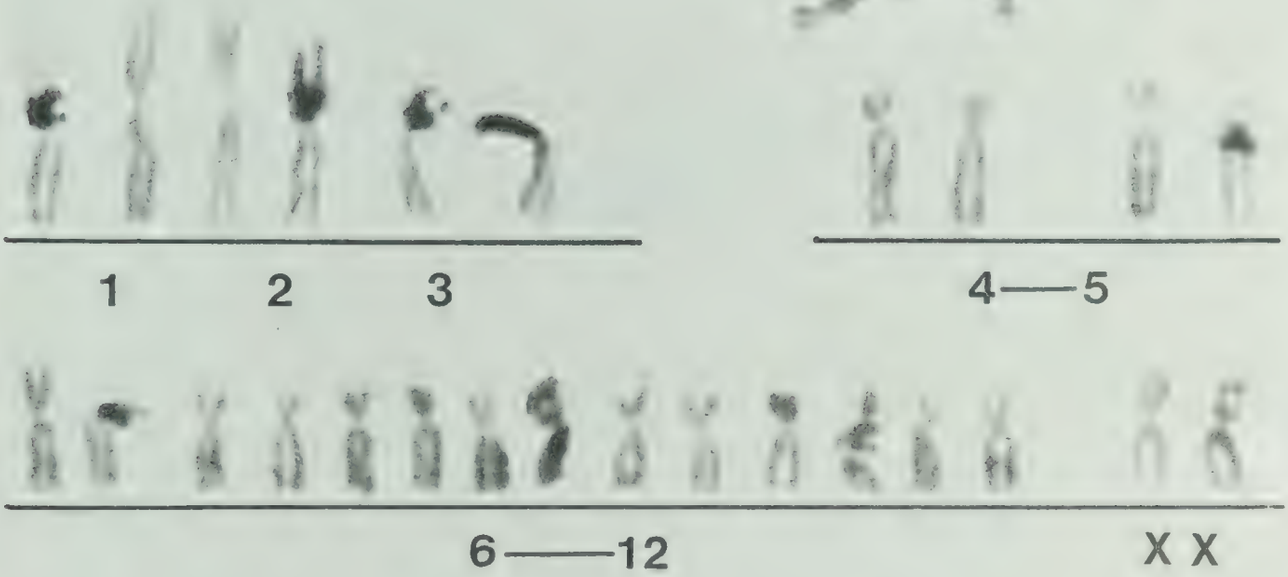
21 — 22

X 2800

10 µ

X 1700





1 2 3

4—5

6——12

X X

13——15

16——18

19——20

21——22

X 2800

10 µ

APPENDIX II

The Kinetics of the Surviving Lymphocyte under Radiation Stress: a Biological Dosimeter.

For some time it has been realized that the lymphocyte is among the most radiosensitive of all body cells and, moreover, that the degree of lymphocyte depression in the peripheral blood after irradiation is dose dependent (Haley, 1965). According to Jacobson et al., (1950) the following data relate to leukocyte depression and recovery in rabbits after acute whole-body X-irradiation:

Dose	Per cent depression		Maximum depression (hrs)		Recovery to normal (days)	
	Lympho-cytes	Granulo-cytes	Lympho-cytes	Granulo-cytes	Lympho-cytes	Granulo-cytes
24	25	-	24	-	2	-
50	25	-	48	-	16	-
100	50	-	48	-	36	-
300	74	-	24	-	50	-
500	90	50	48	72	50	9
600	90	75	48	96	50	9
800	90	90	72	96	50	23

As can be seen, the most pronounced effect on the peripheral blood leukocyte series after X-irradiation is on the lymphocytes, which not only decrease in number but tend to disappear altogether within the first 48 hours post radiation (p.r.). Therefore, the decrease in lymphocytes in the circulating blood is regarded as the most sensitive index of radiation damage (Warren, 1954). Although a

considerable amount of data relate to the depression of peripheral lymphocytes in organisms other than humans, as Mathé (1965) points out, cellular disorders, following a given dose of homogeneous external irradiation with X-rays, are not precisely identical from one species to another. Nevertheless, these disorders run "a course that is more or less the same for most species, races, strains or individuals, in any case among mammals" (p. 276). It has to be pointed out, however, that experiences with single dose exposures of ionizing radiation to humans are extremely rare (Küss et al., 1962; Brucer, 1961) and consequently most of the information available relates to changes of the haematopoietic system after exposure to a fractionated or a chronic dose of ionizing radiation as applied in therapeutic X-ray procedures (Tubiana, 1967).

From the limited information available it appears that an acute dose of 100 to 200 R results in a maximum lymphocyte depression of 50% after 24-48 hrs, with a return to normal before 36 days post irradiation (Lawrence et al., 1948; Cronkite, 1949; Jacobson et al., 1949). According to Alexander (1959), a dose of 300 R results in a lymphocyte depression of approximately 80% (Figure 1). Doses as low as 250 mrem of X-irradiation cause a temporary reduction in lymphocyte count (U.N.S.C.E.A.R, 1958). Although most sources quote 50 days as the recovery time for lymphocyte depression (Errera and Forssberg, 1960), recently Buckton et al., (1967) have found recovery times in excess of 1000 days.

The profound initial decrease in the lymphocyte count after exposure to ionizing radiation has lead in the past to erroneous conclusions with regard to the lifespan of the lympho-

cyte. Clemedson and Nelson (1960) pointed out that "the mean lifetime of the circulating lymphocytes has been determined to be from hours to 1 to 2 days". Consequently, the initial fall in lymphocyte count after an acute exposure to ionizing radiation could therefore be explained on the basis of mitotic delay and short half life of the lymphocyte. Due to the work of Buckton et al., (loc. cit.), we are now informed that a certain class of lymphocytes are estimated to have a mean life span of about 1600 days. In the light of this estimate, the initial decrease in lymphocyte count within 48 after irradiation can no longer be explained as being due to an extremely short life span of the lymphocyte and mitotic delay. The present authors therefore have assumed that the lymphocyte constitutes a multi-unit target which on receiving multiple hits is recognized and preferentially removed from the peripheral blood. Although mitotic delay may enhance the sudden and pronounced decrease of the numbers of these cells in the peripheral blood, it is not believed that at relatively low dose levels this delay is of significance.

The mechanism of recovery after the initial 48 hours p.r. not only involves the increased production of lymphocytes in tissues outside the irradiated volume and the recovery of lymphopoietic centres within this volume (Buckton, et al., loc. cit.), but also the removal of lymphocytes which although impaired, were not preferentially removed from the peripheral blood immediately after irradiation. On the basis of the damage received these latter lymphocytes do not qualify for preferential removal within 48 hours p.r. and are replaced at a later time according to their reduction

in life span. Under in vitro conditions (without the presence of a mitotic stimulator) it can be expected that irradiated surviving lymphocytes will decrease in two stages:

(1) Early reductions i.e., death of severely impaired lymphocytes -

(2) Late reduction i.e., death of impaired lymphocytes -

Under in vitro conditions, without the presence of a mitotic stimulator, the life span of the non-irradiated lymphocyte is approximately 7-10 days and it is therefore evident that the in vitro technique enables an estimate of early and late reduction within a relatively short observation time.

On the basis of the long mean life span of the lymphocyte it can be expected that under in vivo conditions a linear relationship exists between lymphocyte count and time after 48 hours p.r. Although the decay of impaired lymphocytes (late reduction) with time is exponential (Buckton et al., loc. cit.) (due to the relatively long life span of the lymphocyte,) the resulting decay curve can be treated as being linear over relatively short time intervals.

In constructing a biological radiation dosimeter one has to bear in mind that its use mainly concerns the dose assessment of radiation accidents. In our own experience all radiation accidents have in common the fact that the biological assessment has to be carried out after the physical dosimeter (film badge) has registered such an accidental exposure. Since film badges are periodically recalled for processing, the detection of the accidental exposure to ionizing radiation may be made several weeks post-irradiation. To be useful, a biological radiation dosimeter should

therefore allow, by means of extrapolation, an assessment of the biological damage at the time of the accident and consequently of the dose received.

Since lymphocytes can be conveniently cultured and are, moreover, very radiosensitive, lymphocytes are the obvious choice for an assessment of biological damage due to acute radiation exposure. The principle of such a dosimeter constitutes a comparison between the survival of lymphocytes of the victim *in vitro* and the lymphocyte survival of peripheral blood cultures obtained from volunteers and irradiated at different but known dose levels.

In calculating the dose received at 0 hours it has to be assumed that *in vivo*, there is a linear relationship between recovery in terms of surviving lymphocytes and time. Consequently, the slope of the curve for surviving lymphocytes *vs.* time can be calculated by subsequent survival rate determinations at given time intervals (usually 1 week). Extrapolation of such a survival curve to 0 hour would provide an estimate of the dose received in terms of total body radiation. Over recent years a standard curve involving 265 volunteers was developed by us (Figure 2) and although useful in dose assessment of radiation accidents, one has to bear in mind that at dose levels exceeding 50 R a too low a dose estimate will result. This discrepancy is due to the fact that during the first 48 hours p.r. a considerable number of lymphocytes are preferentially removed by the body and consequently radiation assessment after 48 hours p.r. is carried out on residual bio-

logical damage to the lymphocyte.

In order to account for the initial loss of lymphocytes during the first 48 hours p.r., it has been assumed that this loss concerns cells which have received several hits and are considerably impaired in function and morphology. It is assumed further that the life span of these severely damaged cells in culture is short. Residual damage to lymphocytes of peripheral blood will therefore manifest itself at a later stage during culturing. On the basis of these assumptions lymphocytes from volunteers have been irradiated in vitro at different but known levels of radiation (0, 25, 50, 100, 200, 300, and 400 R) and examined after 0, 24, 36, 48 and 72 hours of incubation for lymphocyte survival rates (Figure 3).

Table I

Percent surviving lymphocytes irradiated in vitro at different dose levels and harvested after different culture times.

Culture time	Dose 0 R	50 R	100 R	200 R	300 R	400 R
0	100	101	100	102	103	100
24	100	96	97	81	52	48
36	100	98	91	78	57	41
48	100	96	90	75	54	42
72	100	86	70	43	17	3
	100*	82*	73*	38*	15	3

* Standard curve values.

It has been noted that the reduction of lymphocyte survival is characterized by two processes. During the first 24 hours a reduction of lymphocytes takes place (i.e. early reduction) especially

at dose levels in excess of 100 R. During the subsequent 24 hours of culture time no further decrease can be noted and consequently the lymphocyte survival curves for 24, 36 and 48 hours are the same for all the dose levels studied (see Table I). During the culture interval from 48 hours to 72 hours a further decrease of lymphocyte can be seen at all dose levels (i.e., late reduction). Hence, from the data obtained from these lymphocyte cultures, three curves can be constructed:

(1) lymphocyte decrease (in per cent) after 48 hours of incubation (Early reduction);

(2) lymphocyte decrease (in per cent) after 72 hours of incubation and,

(3) lymphocyte decrease (in per cent) occuring over 24 hours starting with the 48th hour and ending with the 72nd hour of incubation (Late reduction).

For an exposure of 200 R, the early reduction amounts to 25% of the total number of lymphocytes whereas at 300 R this percentage has increased to 46% and at 400 R to 58%. These figures compare reasonably well with experimental figures obtained for the reduction of the lymphocyte count of known radiation cases during the first 48 hours p.r. Therefore, it is assumed that the early reduction of lymphocyte survival during the first 48 hours of incubation of *in vitro* radiated lymphocytes constitutes severely damaged cells which in an *in vivo* system would have been preferentially removed by the body.

On the basis of the above assumption, the standard lymphocyte survival curve as derived from *in vitro* studies on volunteer

blood samples, can be corrected in such a manner that this curve relates to late reduction only (Figure 4). With the aid of the corrected standard lymphocyte survival curve biological damage to lymphocytes of a radiation victim can be assessed providing blood sampling is carried after 48 hours p.r. In case assessment can be performed immediately following acute exposure to ionizing radiation, the total reduction curve (0-72 hours) should be employed for lymphocyte survival rate of comparison.

J. WEIJER

References

Alexander, P., Atomic Radiation and Life, p. 94 Penguin, Whitefriars Press, London, 1959.

Brucer, M., Int. J. Appl. Radiat. 10, 99-105 (1961)

Buckton, K.E., Brown, W.M.C., Smith P.G. Nature 214, 470 (1967)

Clemedson, C.-J, and Nelson, A., In "Mechanisms in Radiobiology V II Chapter 2. (M. Errera and A. Forssberg, eds.) Academic Press, London, 1960

Cronkike, E.P., J. Am. Med. Assoc. 139, 366 (1949)

Errera, M. and Forssberg, A., Mechanisms in Radiobiology, V II. Academic Press, London, 1960

Haley, T.J., In "Nuclear Haemotology" (E. Szirmai, ed.) Chapter 10, Academic Press, London, 1965

Jacobson, L.O., Marks, E.K., and Lorenz, E. Radiology 52, 371 (1949)

Jacobson, L.O., Simmons, E.L., Bethard, W.F., Marks, E.K., and Robson, M.J. Proc. Soc. Exptl. Biol. Med. 73, 455-459 (1950)

Küss, R., Legrain, M., Mathé, G., Nedey, R., Camey, M., Tabiana, M., Lalanne, C., Schwarzenberg, L., Larrieu, M.J., Maisonnet, M., Busset, F., and Delareau, P., Rev. Franc. Études Clin. Biol. 7, 1028 (1962)

Lawrence, J.S., Dowdy, A.H., and Valentine, W.N., Radiol. 51, 400 (1948)

Mathé, G., In "Nuclear Haematology" (E. Szirmai, ed.) Chapter 11, Academic Press, London, 1965.

Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, New York, 1958.

Tubiana, M., I A E A Panel Proceedings, p. 87-99. Vienna, 1967.

Warren, S., Physiol. Rev. 24, 225, (1954)

Figure 1. Effect of a single dose of X-Rays of about 300 R on the different cells in the blood.

(After Alexander, 1959)

Figure 2. Effect of increasing single doses of X-Rays on incidence of surviving lymphocytes *in vitro* at 72 hr. post-radiation.

(After Weijer, 1969)

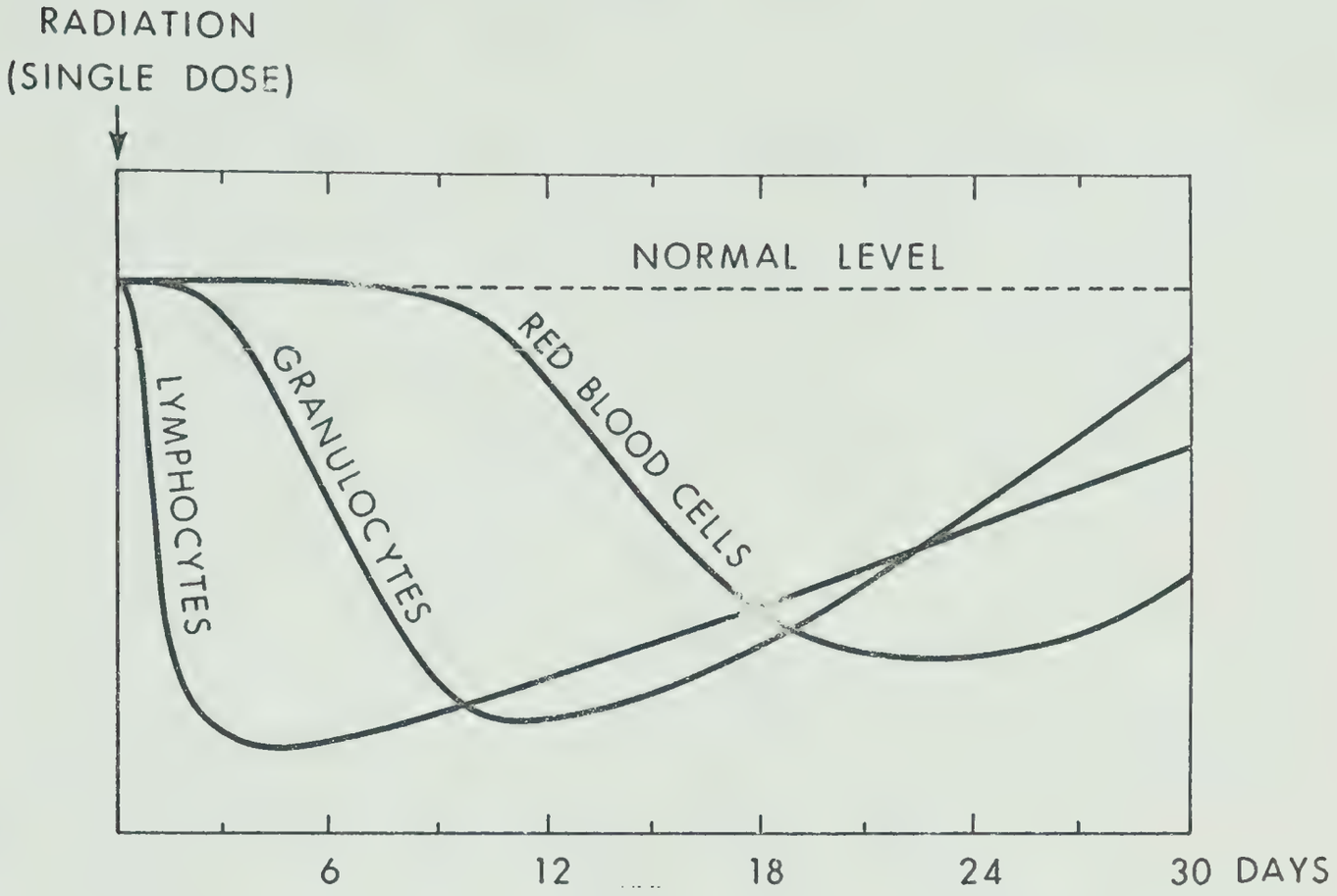

RADIATION
(SINGLE DOSE)
NORMAL LEVEL
RED BLOOD CELLS
GRANULOCYTES
LYMPHOCYTES
6
12
18
24
30 DAYS

Fig. 1

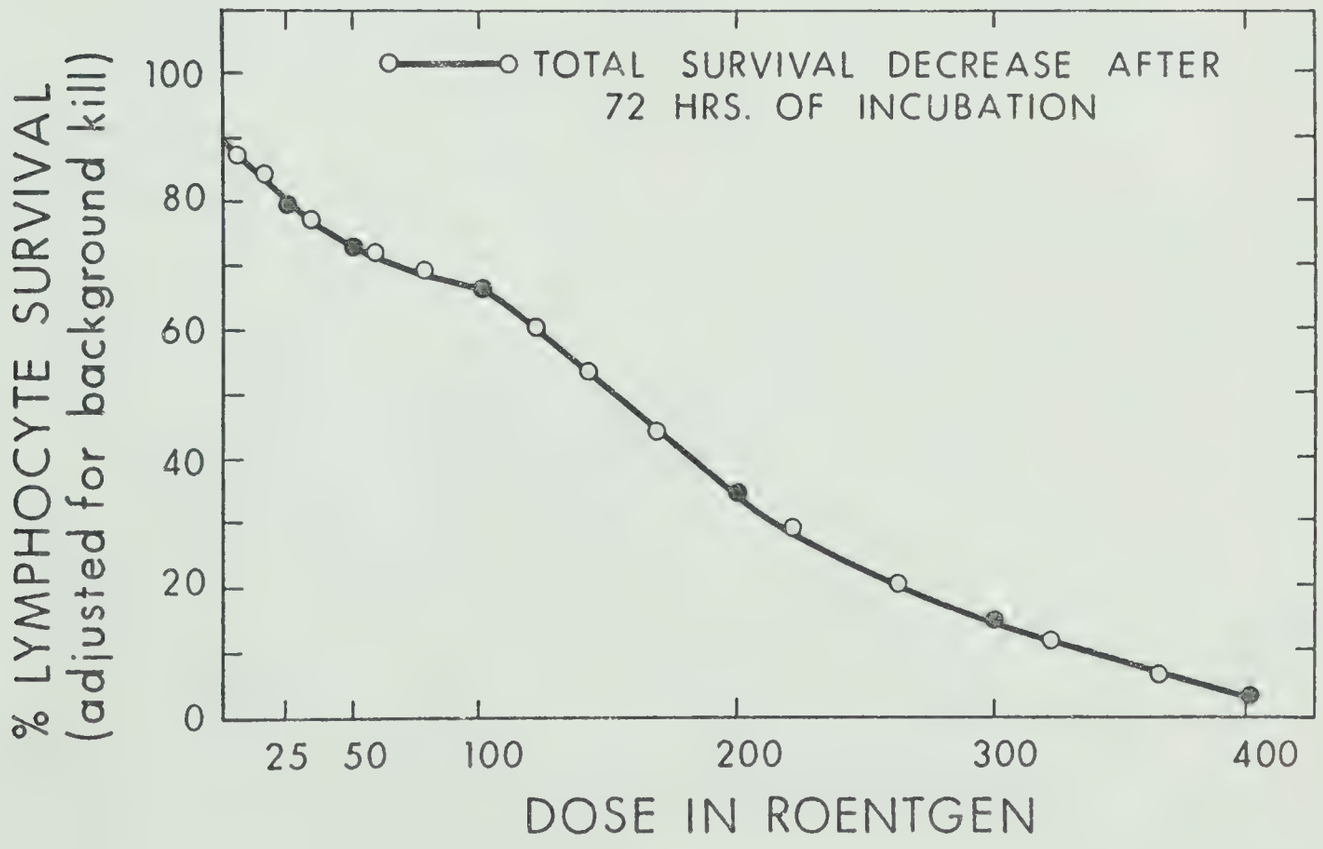

TOTAL SURVIVAL DECREASE AFTER
72 HRS. OF INCUBATION
% LYMPHOCYTE SURVIVAL
(adjusted for background kill)
100
80
60
40
20
0
25
50
100
200
300
400
DOSE IN ROENTGEN

Fig. 2

Figure 3. Effect of single graded doses of X-Rays on incidence of surviving lymphocytes *in vitro* at various times post-radiation.

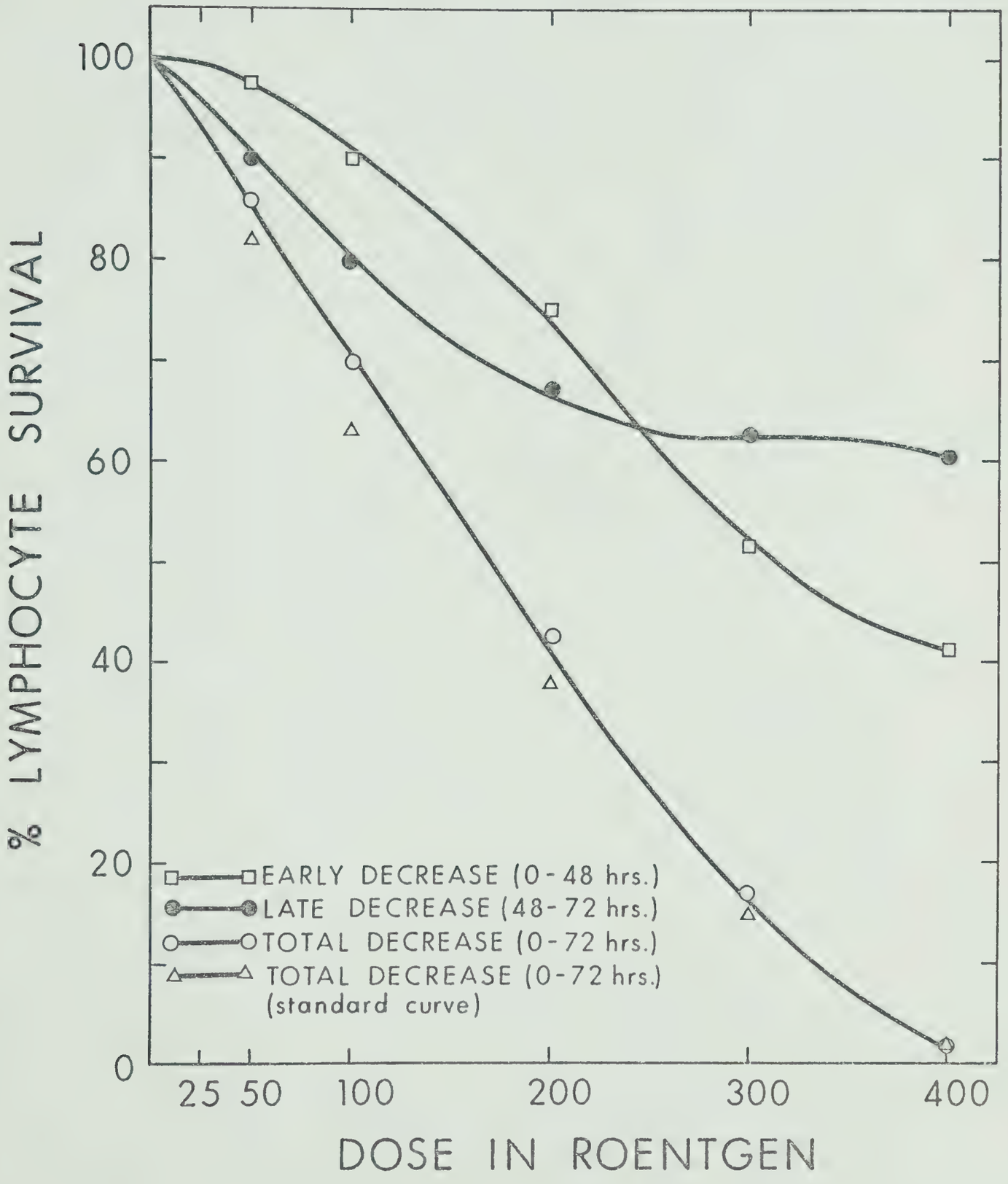
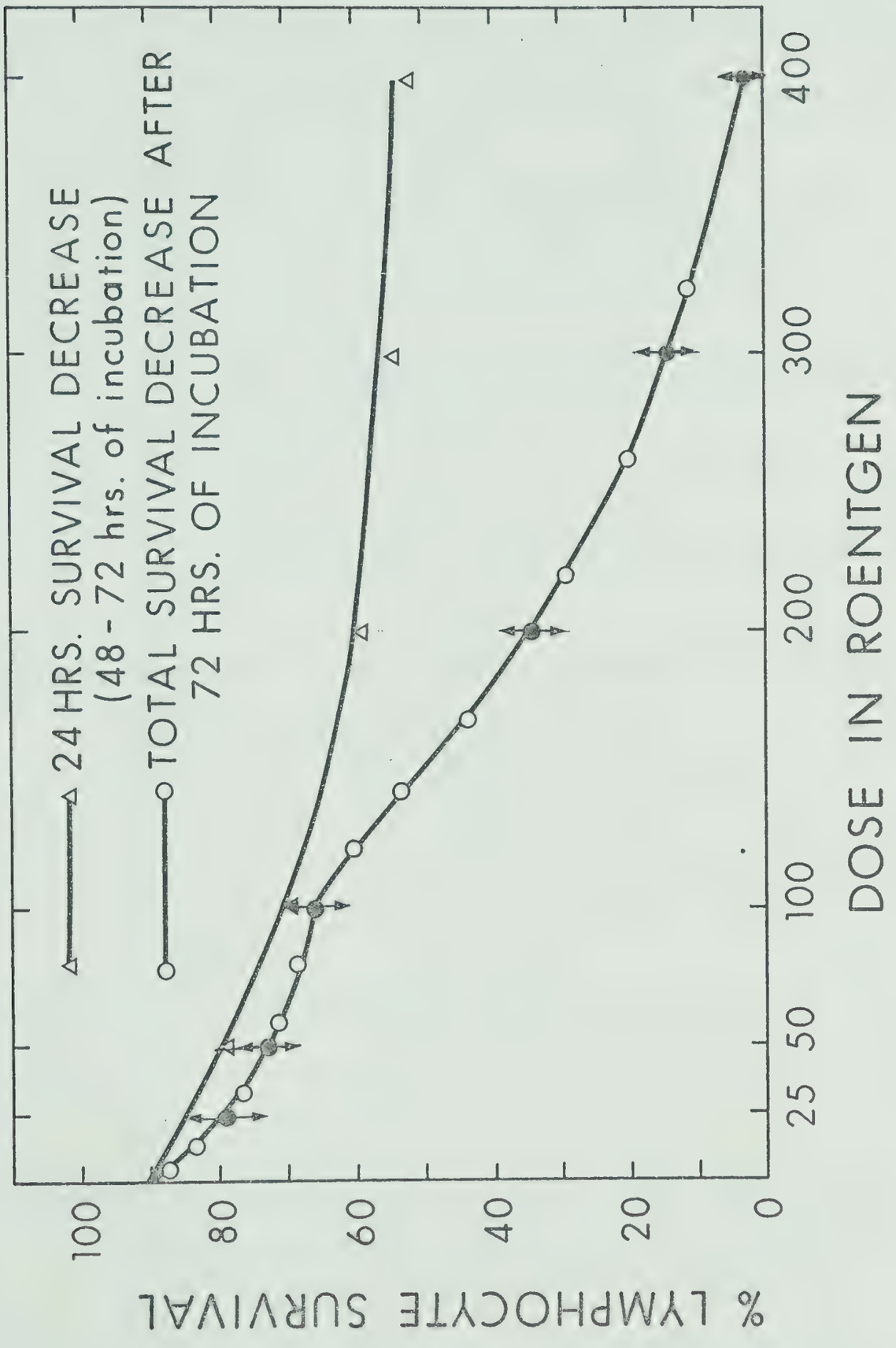

% LYMPHOCYTE SURVIVAL
100
80
60
40
20
0
25 50 100 200 300 400
DOSE IN ROENTGEN
EARLY DECREASE (0-48 hrs.)
LATE DECREASE (48-72 hrs.)
TOTAL DECREASE (0-72 hrs.)
TOTAL DECREASE (0-72 hrs.)
(standard curve)

Figure 4. Effect of single graded doses of X-Rays on incidence of surviving lymphocytes *in vitro*. Correction of standard survival curve to relate to the late decrease phenomenon.


24 HRS. SURVIVAL DECREASE
(48 - 72 hrs. of incubation)
TOTAL SURVIVAL DECREASE AFTER
72 HRS. OF INCUBATION
% LYMPHOCYTE SURVIVAL
100
80
60
40
20
0
25 50 100 200 300 400
DOSE IN ROENTGEN

BIBLIOGRAPHY

Bell, A.G., and D.G. Baker, 1962. Irradiation-induced chromosone aberrations in normal human leukocytes in culture. Can. J. Genet. Cytol. 4: 341-351.

Bender, M.A., 1964. Chromosome breakage in vitro. Mammalian Cytogenetics and Related Problems in Radiobiology. Pergamon Press, New York.

Bender, M.A., 1968. Somatic chromosomal aberrations. Arch. Environ. Health 16: 556-564.

Bender, M.A., and M.A. Barcinski, 1968. Kinetics of two-break aberration production in human leukocytes. Science(unpublished report).

Bender, M.A., and P.C. Gooch, 1962a. Types and rates of X-ray induced chromosome aberrations in human blood irradiated in vitro. Proc. Natl. Acad. Sci. 48: 522-531.

Bender, M.A., and P.C. Gooch, 1963. Chromatid-type aberrations induced by X-rays in human leukocyte cultures. Cytogenetics 2: 107-116.

Bender, M.A., and D.M. Prescott, 1962. DNA synthesis and mitosis in cultures of human peripheral leukocytes. Exp. Cell Res. 27: 221-229.

Berke, H.L., G.H. Wilson, and E.S. Berke, 1969. Size distribution changes in peripheral lymphocytes of the rat after X-irradiation. Radiation Res. 37: 181-191.

Bloom, W., and D.W. Fawcett, 1962. A Textbook of Histology (Eighth Ed.). W.B. Saunders Co. Philadelphia.

Buckton, K.E., and M.C. Pike, 1964. Chromosome investigations on lymphocytes from irradiated patients: Effect of time in culture. Nature 202: 714-715.

Buckton, K.E., and M.C. Pike, 1964. Time in culture: An important variable in studying *in vivo* radiation induced chromosome damage in man. Int. J. Rad. Biol. 8: 439-452.

Caron, G.A., 1968. Fate of transformed human lymphocytes induced by PHA and antigens *in vitro*. Nature 218: 1053-1055.

Chernick, B., Personal communication.

Cooper, E.H., P. Barkhan, and A.J. Hale, 1963. Observations on the proliferation of human leukocytes cultured with phytohaemagglutinin. Brit. J. Haemat. 9: 101-111.

Coulson, A.S., and D.G. Chalmers, 1964. Separation of viable lymphocytes from human blood. Lancet 1: 468-469.

Dienstbier, Z., M. Arient, and J. Pospisil, 1967. Post-irradiation changes in the bone marrow and peripheral blood. In "Effects of Ionizing Radiation on the Maematopoietic Tissue". Vienna Panel Proceedings, IAEA, Vienna, pp. 60-68.

Dobson, R.L., and M.M. Chupp, 1957. Effects of small measured doses of radiation on lymphocyte morphology in men. Proc. Soc. Exp. Biol. N.Y. 95: 360-361.

Everett, N.B., R.W. Caffrey, and W.O. Rieke, 1964. Radioautographic studies of the effect of irradiation on the long lived lymphocytes of the rat. Radiat. Res. 21: 383-393.

Ferguson-Smith, M.A., and S.D. Handmaker, 1963. The association of satellited chromosomes with specific chromosomal regions in cultured human somatic cells. Ann. Hum. Genet. Lond. 27: 143-156.

Frank, J.A., and T.F. Dougherty. 1953. Evaluation of susceptibility to stress stimuli determined by "stress" lymphocytes. Federation Proceedings 12: 45-46.

Gowans, J.T., 1959. The life history of lymphocytes. Brit. Med. Bull. 15: 50-53.

Hamilton, L.D., 1959. In "Kinetics of Cellular Proliferation" (F. Stohlman, ed.). Grune & Stratton, New York. p. 151.

Hungerford, D.A., A.T. Donnelly, P.C. Nowell, and S. Beck, 1959. The chromosome constitution of a human phenotypic intersex. Am. J. Hum. Genet. 11: 215-236.

Ingram, M., 1960. The occurrence and significance of binucleate lymphocytes in peripheral blood after small radiation doses. In "Immediate and Low Level Effects of Ionizing Radiations". Venice Symposium.

Ingram, M., and S.W. Barnes, 1951. Experimental confirmation of a previously reported unusual finding in the blood of cyclotron workers. Science 113: 32-34.

Jacobson, C.B., 1968. In "Genetics in Medical Practice" (E. Bartalos, ed.) J.B. Lippincot Company, Philadelphia, pp. 101-122.

Mathé, G., 1965. In "Nuclear Haematology" (E. Szirmai, ed.). Academic Press, New York. pp. 275-388.

Mathé, G., and J. Bernard, 1960. In "Lésions provoquées par les radiations ionisantes" (J. Delarue and L. Frühling, eds.) Masson, Paris. p. 28.

Moorhead, P.S., P.C. Nowell, W.J. Mellman, D.M. Battips, and D.A. Hungerford, 1960. Chromosome preparations of leukocytes cultures from human peripheral blood. Exp. Cell Res. 20: 613-616.

Neff, R.D., and B. Cassen, 1968. Relative radiation sensitivity of circulating small and large lymphocytes. J. Nuclear Medicine 9: 402-405.

Norman, A. 1967. Multihit Aberrations. In "Human Radiation Cytogenetics" North-Holland, Amsterdam.

Norman, A., and M.S. Sasaki, 1966. Chromosome-exchange aberrations in human lymphocytes. Int. J. Radiat. Biol. 11: 321-328.

Norman, A., R.E. Ottoman, M. Sasaki, and R.C. Veomett, 1964. The frequency of dicentrics in human leukocytes irradiated *in vivo* and *in vitro*. Radiology 83: 108-110.

Norman, A., M.S. Sasaki, R.E. Ottoman, and A.G. Fingerhut, 1966. Elimination of aberrations from human lymphocytes. Blood 27: 706-713.

Nowell, P.C., 1960. Phytohaemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. Cancer Res. 20: 462-466.

Ottesen, J., 1954. On the age of human white cells in peripheral blood. Acta. Physiol. Scand. 32: 75-93.

Robbins, J. H., 1960. Tissue culture studies of the human lymphocyte. Science 146: 1648-1654.

Sasaki, M.S., and A. Norman, 1966. Proliferation of human lymphocytes in culture. Nature 210: 913-914.

Sasaki, M.S., and A. Norman, 1967. Selection against chromosome aberrations in human lymphocytes. Nature 214: 502-503.

Schrek, R. 1961. Qualitative and quantitative reactions of lymphocytes to X-rays. Ann. N.Y. Acad. Sci. 95: 839-848.

Shiels, D.O., 1962 Effects on lymphoid cells of occupational exposure to low doses of ionizing radiation. Aust. Ann. Med. 11: 267-269.

Trowell, O.A. 1953. The sensitivity of lymphocytes to ionizing radiation. J. Pathol. Bacteriol. 64: 687-704.

Weijer, J. 1969. Report in preparation.

Weijer, D., H.E. Duggan, and J. Weijer, 1963. Assessment of radiation damage by means of peripheral blood cultures. J. Can. Assoc. Radiol. 14: 60-66.

Weijer, J., and D. Weijer, 1968. Radiation Accident Case L.S. Report to the Workmens Compensation Board, Department of Health and Welfare, Alberta, Canada.

Weijer, J., and S.H. Weisberg, 1966. Karyokinesis of the somatic nucleus of Aspergillus nidulans. I. The juvenile chromosome cycle (Feulgen Staining). Can. J. Genet. Cytol. 8: 361-374.

Yoffey, J.M. 1962. The present status of the lymphocyte problem. Lancet 1: 206-211.

B29928